THE JOURNAL OF PHYSIOLOGY

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J. N. LANGLEY, Sc.D., LL.D., F.R.S.

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Vol. LIV.

1920—1921

CAMBRIDGE UNIVERSITY PRESS
C. F. CLAY, Manager
LONDON: FETTER LANE, E.C. 4

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R67

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THE RECOVERY PROCESS OF EXCITABLE TISSUES.

Part I. BY E. D. ADRIAN, M.D.

(*From the Physiological Laboratory, Cambridge.*)

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IN 1912 Keith Lucas and I (1) investigated the course of recovery in a nerve fibre after the passage of an impulse and we were able to map out the return of the two main functions of conductivity and excitability. We found that the nerve returned gradually to normal during the relative refractory period and then passed through a stage in which its excitability and its power of conduction were increased above their normal value. We called this stage of recovery the "supernormal period," and we showed that its existence might be of great importance in the mechanism of the central nervous system, since it might account for the type of nervous summation in which a series of impulses succeed in passing a region of imperfect conduction whereas a single impulse fails to do so. The supernormal rise of excitability was present in all our experiments, but it was never very great and it showed considerable variation from one preparation to another. We did not seek the cause of these variations since we were concerned with the mechanism of summation and not with the nature of the recovery process.

The present investigation is an attempt to carry the analysis a stage further, to discover what factors account for the observed variations in the supernormal phase and if possible to suggest some explanation of its existence. I was all the more anxious to undertake it because in the course of some later work I had observed much greater variations in the supernormal excitability than those mentioned in our paper. In fresh preparations the rise of excitability was often absent, or at any rate too small to detect, whilst in preparations which had been kept in Ringer's fluid for twelve hours or more the supernormal excitability was sometimes as great as 115 p.c. of the resting value.

Moreover the existence of the supernormal phase of recovery was itself a puzzle. It seemed that the recovery process overshot the mark before the tissue settled down to its resting condition and such a proceeding did not suggest any simple process such as the diffusion away of the products of activity or the re-establishment of equilibrium by a chemical reaction. It seemed possible that a knowledge of the factors which determined this apparent overthrow would go some way towards elucidating the whole mechanism of recovery.

At the beginning of the investigation it was thought that the variations in the form of the recovery curve might be due to variations in the concentration of calcium ions with which the tissue was in equilibrium. Lucas had found a progressive change in the excitability constants of certain tissues and he had shown that this was due to a lack of equilibrium between the concentration of calcium in the tissues and that in the surrounding fluid. The progressive change in the recovery curve in some preparations suggested the same cause. It was however very soon clear that the concentration of calcium ions had little or nothing to do with the supernormal phase and that the only important factor was the concentration of that ion which has already shown itself to be of paramount importance in so many biological processes, namely the hydrogen ion. How much the form of the recovery curve depends on the H˙ ion concentration will appear in the following sections.

I. THE INFLUENCE OF H˙ ION CONCENTRATION ON THE RECOVERY PROCESS.

(a) *The recovery of excitability in nerve.*

Method. In the following experiments the nerve of a sciatic gastrocnemius preparation of the frog was perfused[1] with a buffered Ringer's fluid of known $H^{\cdot}$ ion concentration and the course of recovery was mapped out by the method already described by Lucas and Adrian(1). The muscle nerve preparation was set up in the vulcanite chamber shown in Fig. 1. The muscle and the distal part of the nerve lie in a bath of neutral Ringer's fluid and the proximal part of the nerve is bathed by a slow stream of Ringer's fluid of required P_H from one of three Mariotte bottles connected to the inflow tube. The nerve lies in a series of chambers which are completely filled with the perfusing fluid. The chambers are closed above by a glass plate and the point of exit of the nerve is sealed with a plug of vaseline so that the perfusing fluid shall not come into contact with the nerve ending or the muscle.

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[1] It is perhaps incorrect to talk of perfusing a nerve when the fluid surrounds it, but no other word will make it clear that the fluid is continually renewed.

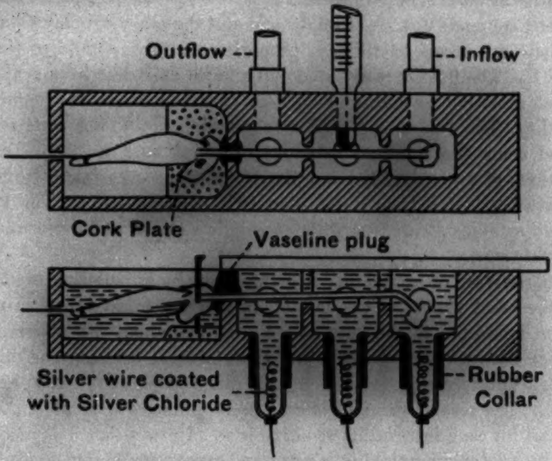


Fig. 1. Chamber with non-polarisable electrodes for perfusing nerve with fluids of different $H^{\cdot}$ ion concentration.

The stimulating current is led into the chamber by a series of non-polarisable electrodes of the type devised by Lapicque(2). Each electrode consists of a small glass tube filled with Ringer's fluid and containing a spiral of fine silver wire sealed in with wax. Before the electrode is used, a thin coating of silver chloride is deposited electrolytically on the wire by connecting it to the positive pole of a two volt accumulator. The negative pole is connected to a platinum cathode dipping into the fluid in the chamber. The current is passed for two minutes and the fluid is changed before the preparation is set up. These electrodes show no polarisation after several days' work and it is a simple matter to renew the coating of silver chloride from time to time. They have the advantage of a low resistance and it is never necessary to dismantle them for cleaning or recharging. The current is led through the fluid from these electrodes and takes effect on the nerve at one of the slots where the density of current flow is suddenly increased.

The fluid used was that employed by Mines for work on the heart (3). Besides the ordinary ingredients of Ringer's fluid it contains small quantities of boric acid and sodium acetate to act as buffers. Two stock solutions are made up, one strongly acid and the other strongly alkaline, and these are mixed in different proportions to give a fluid of the required P_H. The fluid is easily made up and its only drawback is that it is most sensitive to additions of acid or alkali about the region of neutrality. The P_H of the fluid was determined by the colorimetric method using the standard solutions of Clark and Lubs (4). During the course of an experiment samples of the fluid were taken from the Mariotte bottle and from the outflow tube of the chamber. The P_H of the two samples showed no difference unless the rate of perfusion was extremely slow.

The recovery of excitability is mapped out by stimulating the nerve with the break shocks of two induction coils which have their primary circuits connected to the keys of a Lucas pendulum. By altering the distance between the two keys the time interval between the stimuli can be accurately adjusted over a wide range (from about ·0001 sec. to ·1 sec.). The second stimulus is sent in at different intervals after the first and for each interval its strength is so adjusted that it is just able to set up a second impulse in the nerve and cause a summated contraction in the muscle instead of a single twitch. The strength of stimulus needed to set up an impulse at any stage of recovery varies inversely as the excitability of the nerve at that time. Thus the return of excitability can be mapped out by determining the strength and time of occurrence of the second stimulus which will just suffice to give a summated contraction.

In these experiments the strength of the stimulus was adjusted by varying the resistance in the primary circuit of the coil. As the coils had no iron core the current induced in the secondary would be directly proportional to that in the primary and therefore inversely proportional to the total resistance in the primary circuit. This was controlled by inserting a dial resistance box reading from one to one thousand ohms in series with the primary of the coil and a two volt accumulator. The secondary coil was shifted until the break shock would just excite when the resistance in the primary was about 100 ohms; the secondary was not moved after the preliminary setting and the strength of the shock was altered by adding or subtracting resistances. This method of determining the strength of the stimulus has proved far more rapid and more accurate than the usual method of shifting the secondary coil and working out the strength from a calibration curve. As a rule the secondary circuits were connected so that the two stimuli fell at different points on the nerve, but the exact arrangement made no difference to the result.

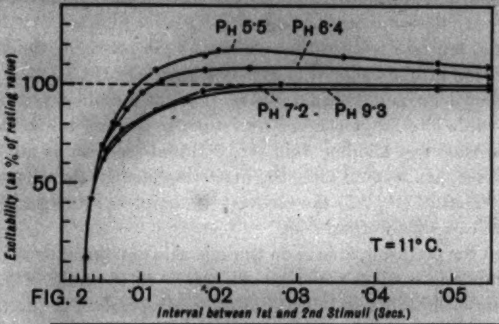


FIG. 2

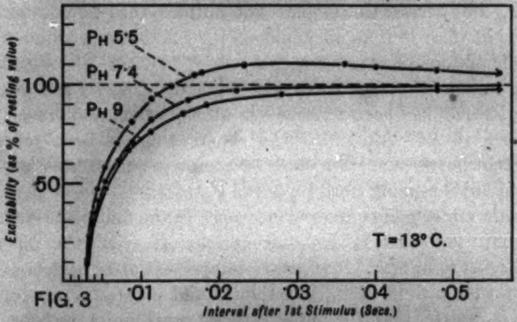


FIG. 3

Figs. 2 and 3. Recovery of excitability in nerve perfused with fluids of different P_H.

The most important outcome of these experiments can be seen at once from Figs. 2 and 3. These show the return of excitability in two preparations perfused with solutions of different H˙ ion concentration. The nerve was perfused for half an hour with each solution before the recovery curve was determined and the normal or resting excitability was measured afresh for each solution. The normal excitability has a different value for fluids of different P_H (the exact significance of these differences will be dealt with later), but in the figures the curves have been drawn so that the normal excitability in each case is given the value 100. The mean error in each determination is about ± 1 p.c.

In Fig. 2 it will be seen that as long as the perfusing fluid is on the alkaline side of neutrality (*i.e.* with a P_H greater than 7) the recovery curve shows no supernormal phase. The excitability returns to its resting value and stays there. The return to normal takes place more slowly in the more alkaline fluid (P_H 9·3) and recovery is not complete until ·06 sec. has elapsed after the first stimulus. In the solution which is nearly neutral (P_H 7·2) the recovery is complete in ·03 sec. but there is no sign of a supernormal rise.

When the perfusing fluid is on the acid side of neutrality the recovery curve shows an obvious supernormal phase. In the fluid of P_H 6·4 the maximum excitability is 109 p.c. of the resting value, in the more acid fluid (P_H 5·5) it rises to 117 p.c. The initial return to normal is much more rapid than it is in the fluid of P_H 7·2, but the whole recovery process is not complete in ·06 sec.

Fig. 3 shows the same effect with fluids of P_H 9, 7·4 and 5·5, and an identical result has been obtained in all the other preparations which have been tested. Altogether the experiment has been repeated on 23 different nerves and from these 56 complete curves have been determined in fluids ranging from P_H 3·8 to P_H 10·7.

Certain minor points deserve mention. In the first place it is difficult to estimate the exact H˙ ion concentration at which the supernormal phase begins to appear. It is invariably present after perfusion for half an hour with a fluid of P_H less than 7 and invariably absent with a fluid of P_H greater than 7·4, but in between these limits the type of curve for a given P_H depends to a considerable extent on the past history of the preparation. If it has been recently perfused with an acid fluid it usually retains the acid type of recovery curve (*i.e.* the curve with a supernormal phase) even after perfusion for half an hour or more with a fluid of P_H 7·4. If it has been in alkali it may retain the alkaline type of curve after perfusion with a fluid of P_H 7, although the alkaline type of curve is changed more easily than the acid. Thus the nerve which is perfused with a fluid near the neutral region tends to retain the form of recovery curve which it had before, and for this reason it is impossible to specify any single $H^{\cdot}$ ion concentration which can be regarded as the equilibrium condition for the appearance or non-appearance of the supernormal phase in the curve. The existence of this neutral zone within which either type of curve may persist for an indefinite time suggests that the change in the nerve fibre brought about by an altered $H^{\cdot}$ ion concentration exhibits something akin to hysteresis. Even with fluids which are well outside the neutral zone the nerve does not respond immediately to a change in P_H. After it has been in a fluid of P_H 6 it may take 10 or 15 minutes perfusion with P_H 8·5 before the supernormal phase disappears. This is not due to any failure to bring about a rapid change in the P_H of the fluid in the perfusing chamber, for the analysis of samples of the fluid leaving the chamber shows that the change may be complete in less than a minute. No doubt the delay is due in part to the slow alteration in the fluid which immediately surrounds each nerve fibre, but it is evidently related also to the phenomena of hysteresis within the neutral zone.

Another point of some interest is that there is no evidence that the supernormal phase of the curve appears only between certain limits of $H^{\cdot}$ ion concentration. A fluid of P_H 3 usually suspends conduction altogether in an hour or more, but up to this concentration the supernormal rise of excitability becomes greater and greater as the acidity increases. Thus in one experiment the perfusing fluid was changed eight times at intervals of about three-quarters of an hour and nine recovery curves were determined. The relation of the supernormal rise of excitability to the P_H of the perfusing fluid is shown below. Evidently the more acid the perfusing fluid the greater is the increase of excitability during the supernormal phase.

Sciatic nerve of frog. Temperature 11° C.

P_H of fluid	Maximum excitability (resting excitability = 100)	Time
(7·1)	(100)	11.15 a.m.
(8·3)	(100)	12 noon
(9·5)	(100)	12.30 p.m.
(7·0)	(100)	1.5
6·4	107	2.0
5·2	115	2.45
(9·5)	(100)	3.30
5·2	112	4.30
4·0	121	5.15

The curves which showed no supernormal rise are placed in brackets.

So far then it has been shown that a nerve in equilibrium with a fluid more alkaline than P_H 7·4 does not pass through any supernormal phase in its recovery after the passage of an impulse. A nerve in equilibrium with a fluid on the acid side of P_H 7 passes through the supernormal phase and this becomes more and more pronounced as the acidity rises. These facts make it easy to explain the variations which Lucas and I observed. In our original investigation we used "neutral Ringer," *i.e.* Ringer's fluid without buffers, and the P_H of this is usually in the neighbourhood of 6·5. Consequently we obtained the supernormal rise in every case. In some later experiments I used Ringer containing small traces of alkali and in these I was unable to detect the supernormal phase. It is interesting to note that in the nerves supplying the claw muscles of Astacus, Lucas (5) found the supernormal excitability as great as 120 p.c., and in these nerves there was a rapid progressive change which led to the failure first of single stimuli and then of all kinds of stimulation. Such a change might well be due to the development of acid in the tissues and this would evidently account for the great supernormal rise.

(*b*) *The recovery of conductivity in nerve.*

Keith Lucas and I found that the supernormal phase of recovery involved an increase of conductivity above the resting value as well as an increase of excitability. An impulse set up during this phase could travel further through a region of decrement than an impulse set up in resting nerve, and for this reason a series of impulses falling at the right intervals might succeed in passing a nerve ending or a synapse when a single impulse would fail. From the point of view of the theory of nervous summation we regarded the increased conductivity during the supernormal phase as a great deal more important than the increased excitability, in fact we dealt with the latter only because it confirmed our view of the existence of a supernormal phase in recovery. Thus it becomes a question of some interest to decide whether the $H^{\cdot}$ ion concentration has the same influence on the recovery of conductivity as it does on that of excitability.

To test this point a nerve was first of all perfused for half an hour with a fluid of known P_H, and a length of about 2 cm. between the electrodes and the muscle was then bathed in a fluid of the same P_H containing in addition 6 p.c. of alcohol. This concentration was great enough to suspend conduction in about half an hour and towards the end of this period the proximal part of the nerve was stimulated alternately with single maximal break shocks and with groups of two shocks separated by an interval of ·024 sec. (this interval corresponds with the maximum rise of excitability in a nerve perfused with an acid fluid at 11° C.).

It was found that if the narcotising fluid was on the alkaline side of neutrality, conduction failed at the same moment for the single and the double stimuli alike. If the fluid was acid there was, as a rule, a period of about 20 seconds within which the two stimuli produced a contraction in the muscle whereas the single stimulus had no effect. It is by no means easy to catch the nerve at the exact moment when conduction fails for a single impulse and therefore in two out of six "acid" experiments the difference between single and double stimuli was not observed. However in four of them the double stimuli continued to affect the muscle after the single stimulus had failed; on the other hand in all the "alkaline" experiments the double and single stimuli failed at the same moment.

In a second series of experiments the use of alcohol as a narcotic was dispensed with and the impaired conduction was brought about by treating a length of nerve with strongly acid or strongly alkaline fluids. A fluid of P_H 2·8 caused failure of conduction for a single stimulus in 83 minutes but the double stimuli still produced a contraction in the muscle for two minutes longer. In another experiment with a Ringer of P_H 2·1 the single stimulus failed after 39 minutes and the double stimuli succeeded for another 50 seconds. On the other hand a fluid of P_H 11·8 caused failure in 23 minutes for single and double stimuli alike and one of P_H 11·4 had the same effect after 63 minutes. In the two "alkaline" experiments the interval between the two stimuli was varied from ·024 to ·08 sec. but none of these intervals led to summation.

These experiments show that in a nerve which has been perfused with an acid fluid it is possible to impair the conduction in such a way that a single impulse fails to reach the muscle, although two impulses may succeed if the second travels in tissue which is in the supernormal phase of recovery. When the nerve is in an alkaline fluid no such effect is observed. Thus the "acid" nerve shows a phase of increased conductivity as well as a phase of increased excitability, whereas both phenomena are absent in the alkaline nerve.

(c) *The recovery process in cardiac muscle.*

At an early stage in the investigation it was thought desirable to extend these results to other excitable tissues besides the sciatic nerve of the frog. Skeletal muscle has the disadvantage that the contractile change outlasts the whole of the recovery process and therefore a stimulus falling during the recovery from a previous disturbance will find the muscle still contracted. The change in shape may cause apparent changes in excitability which will mask the true recovery phenomena. Cardiac muscle is more easily dealt with, because in it the contraction is usually over before recovery is complete and therefore it is possible to measure the excitability in the later stages of recovery without the same liability to error.

The only published determinations of the recovery of excitability in the heart are those of Trendelenburg(6). In his experiments the circulation was intact and the heart was beating spontaneously; the curves show a gradual return to normal excitability without a supernormal phase.

I have repeated these measurements in hearts exposed to fluids of different P_H, paying special attention to the later stages of recovery. For the accurate measurement of this part of the curve it is essential that the heart should not beat spontaneously, as a fresh beat may arrive from the sinus before recovery is complete. It should be possible to overcome this by using a preparation of the isolated ventricle, but in practice it was found difficult to keep such a preparation from beating by itself, or to perfuse it adequately if it did not beat. In the method finally adopted the frog's heart was perfused for half an hour with a fluid of known P_H through the inferior vena cava and at the end of this period a Stannius ligature was applied. This put an end to the perfusion, and the determinations on the quiescent ventricle were carried out as rapidly as possible to minimise the effect of any slight alteration which might take place in the reaction of the fluid remaining in the ventricle. As a matter of fact the response to fluids of different P_H was so constant that it is very unlikely that the condition of the ventricle can have changed rapidly after the ligature was applied. As in the case of nerve fibre, the change brought about by an altered $H^{\cdot}$ ion concentration proceeds very slowly and may not be complete after perfusion for a quarter of an hour; thus a slight change in the P_H of the fluid remaining in the ventricle would be unlikely to have any effect for several minutes at least.

The ventricle was stimulated by break induction shocks from two coils with their primary circuits connected to a rotating contact breaker. Non-polarisable electrodes were used and these were applied on either side of the ventricle midway between base and apex. In estimating the normal, or resting excitability it is important to allow the heart to remain at rest for at least 30 seconds, since the whole recovery process may not be complete for 15 seconds or more. The probable error in each determination is greater than in the case of nerve as the threshold is more variable. The error in most of the determinations is about ± 2 p.c.

The effect of fluids of different P_H may be seen from Fig. 4. This gives the recovery curves from four different hearts perfused with Ringer of P_H 6·2, 6·4, 9 and 9·2. The time scale of Fig. 4 is 200 times as long as that in Figs. 2 and 3, but in other respects the curves for cardiac muscle and for nerve resemble one another very closely. The two hearts which were perfused with fluids of P_H 6·2 and 6·4 show a well-marked supernormal phase and the hearts perfused with P_H 9 and P_H 9·2 show none. The neutral region appears to be slightly more on the alkaline side than it is in the nerve fibre since in two experiments the supernormal phase was just perceptible after perfusion with fluids of P_H 7·6 and 7·8, though it was invariably absent when the P_H was greater than 8. It is impossible to test the effect of fluids more acid than P_H 6 as the ventricle becomes completely inexcitable after half an hour's perfusion.

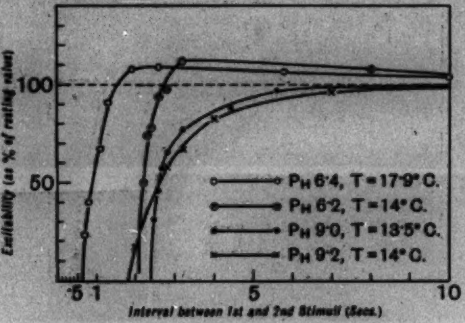
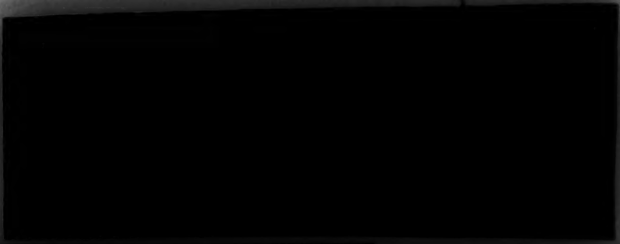


Fig. 4. Recovery of excitability in cardiac muscle.

So far then the results confirm those obtained from the nerve fibre, but the most interesting part of these experiments is that which deals with the recovery of the contractile power in the heart as distinct from its electrical excitability. It has been known since the pioneer work of Bowditch (7) that if the quiescent ventricle is excited by two stimuli separated by a short interval the second contraction may be greater than the first. If the second stimulus falls during the relative refractory period the second contraction is smaller, but as soon as this period is over the second contraction may rise to a greater height and the effect is present with intervals as long as 15 seconds between the first and second contraction. The close agreement between the time relations of this effect and the time relations of the supernormal excitability in the heart suggests that the two may have a common origin, and an inspection of the graphic records obtained after perfusion with different fluids shows that they are certainly very closely related.

The heights of contraction in response to the two stimuli were measured in 18 hearts. Figs. 5 and 6 show the actual tracings obtained in two experiments and Fig. 8 gives the results of four others. The effect of an alteration in the P_H of the perfusing fluid can be seen at once by comparing Fig. 5 with Fig. 6. In Fig. 5 the heart had been perfused for half an hour with a fluid of P_H 8·8 before the Stannius ligature was applied: the second contraction is never greater than the first except in the case of the fifth group of contractions where the height of the first is abnormally small. The interval between the two varies from 2–12 seconds (and intervals up to 20 seconds were tried although these are not recorded in the figure). In Fig. 6 the heart was perfused with a fluid of P_H 6·6 and the second contraction is evidently larger than the

Fig. 5.



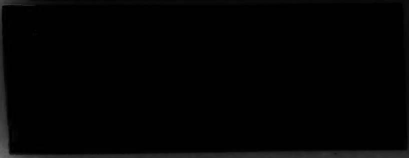


Fig. 6.

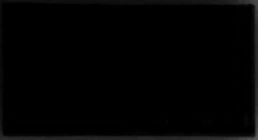


Fig. 7.

Fig. 5. Recovery of contractile power in heart perfused with fluid of P_H 8·8 showing absence of supernormal phase. Time marker beats every two seconds.

Fig. 6. Heart perfused with fluid of P_H 6·6; supernormal phase present. Time marker beats every two seconds.

Fig. 7. Heart perfused with fluid of P_H 6·4. Time marker beating seconds.

(Read from left to right.)

first when the interval between them is greater than 2·8 seconds. The numbers placed over each group of contractions give the interval in seconds between the two stimuli. Fig. 7 (from another heart) shows the form of the two contractions more clearly as the recording surface is moving at a greater rate. Fig. 8 gives a summary of the results in four other hearts: the curves are obtained by comparing the heights of the first and second contraction and giving the first contraction the value 100. The abscissae give the intervals between the first and second contraction and not the intervals between the two stimuli.

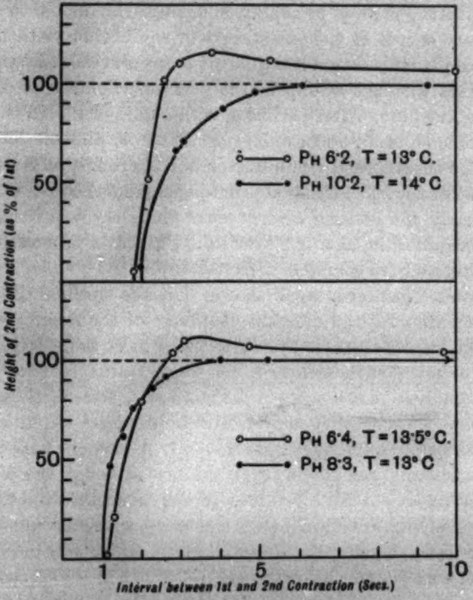


Fig. 8. Recovery of contractile power in hearts perfused with fluids of different P_H.

In the early stages of recovery the second contraction begins before the first has subsided. In this case the height of the second contraction is measured from the point at which it begins to diverge from the first contraction. Thus in the second group in Fig. 6 the height of the second contraction would be taken as 50 p.c. of the first although the summits of both contractions are on a level.

The four curves are in every way comparable with those expressing the return of excitability in the heart or in the sciatic nerve. When the heart had been perfused with a fluid of P_H 8·3 or P_H 10·2 the recovery of contractile power showed no supernormal phase, when the fluid was of P_H 6·2 or P_H 6·4 the supernormal phase was well marked. A similar result was found in the remaining eleven experiments. Evidently the increased contractile power described by Bowditch and others is one expression of the supernormal phase of recovery and like the other phenomena of the supernormal phase it occurs only when the tissue is in equilibrium with an acid solution.

Owing to the method of experiment it was impossible to determine the recovery curves of the same heart in equilibrium with more than one fluid and for this reason it is difficult to say whether the supernormal rise increases with the acidity of the perfusing fluid, as it does in the case of a nerve fibre. After perfusion with a fluid of P_H 5·8 the contractions become so small and so sluggish that it is difficult to make out whether the supernormal rise exists or not. Certainly it is more easily detected when the P_H of the fluid is in the neighbourhood of 6·3, but this is very nearly the limiting acidity with which the ventricular muscle can be in equilibrium as a more acid fluid causes a progressive change leading ultimately to complete inexcitability.

Dale and Thacker(8) have shown that the optimal $H^{\cdot}$ ion concentration varies for the different chambers of the heart and is more acid for the auricle than for the ventricle. I have some rather indirect evidence to show that the critical $H^{\cdot}$ ion concentration for the appearance of the supernormal phase varies in the same way. In several experiments the recovery of excitability and of contractile power were measured at the same time. As a rule they agreed closely, but in one experiment the second contraction was greater than the first although the excitability did not rise above the resting value. It was found that the stimulating electrodes had slipped down to the auriculo-ventricular junction, and it was therefore possible that the excitability which was measured was that of the auricle and not of the ventricle. When the electrodes were replaced on the sides of the ventricle the recovery of excitability gave the usual supernormal phase. The heart had been perfused with a fluid of P_H 6·8 and this might well have been on the acid side for the ventricle and on the alkaline side for the auricle. The experiment was not repeated and as it stands it can only be regarded as suggesting a possible difference in the critical concentrations for ventricle and auricle.

(d) *The staircase effect in skeletal muscle.*

The staircase effect or "Treppe" in skeletal muscle is generally regarded as having the same mechanism as the Bowditch effect in the heart, although it persists for a very much longer time in skeletal muscle in spite of the shorter duration of the refractory period. If the two phenomena are related we should expect to find the staircase present only when the muscle is perfused with an acid fluid. To test this point the frog's sartorius muscle was set up in a perfusion chamber and stimulated by break induction shocks recurring at intervals varying from one to three seconds. The rate of stimulation was controlled by a rotating contact breaker and the stimuli took effect on the nerve-free pelvic end of the muscle.

In the first series of experiments the contractions were recorded by a very light tension lever with a small moment of inertia. Under these conditions the staircase effect was never observed at all although the muscle had been perfused for four hours with fluids varying from P_H 10 to P_H 4. With repeated stimulation the contractions diminished in height, but after a rest of from 1–10 minutes renewed stimulation did not give rise to the staircase phenomenon and the muscle could be fatigued to complete exhaustion without showing any signs of it.

The staircase however did appear when the tension lever was discarded and an isotonic lever used in its place, and the effect was more easily produced when the moment of inertia was increased by moving the weight further from the axis of the lever. The staircase then appeared when stimulation was renewed after a short pause. Its appearance bore no relation to the P_H of the perfusing fluid between the limits of P_H 10 and P_H 4 and it seemed to depend entirely on the state of fatigue of the preparation.

Thus in its reaction to different concentrations of $H^{\cdot}$ ions the staircase of skeletal muscle behaves quite differently to the summation effect in the heart. This is very easily understood if we take into account Fröhlich's work on the cause of the staircase (9). He finds that it depends entirely on the slower time relations of the contractile response induced by fatigue. The first stimulus after a pause finds the muscle rested, and the contraction and relaxation are rapid; after a few stimuli the onset of fatigue prolongs the rate of contraction and relaxation without, at first, diminishing the force of contraction. With a heavy recording lever this prolongation by itself may be enough to cause an apparent increase in the height of the contraction, and the experiments recorded above suggest that the whole effect often depends on the inertia of the recording apparatus. However Fröhlich shows that the increased duration of the contraction may lead to an actual increase in the tension exerted by the whole muscle, because the longer the duration the more chance is there that all parts of the muscle will be fully contracted at the same moment. Fröhlich's records show clearly that in skeletal muscle the increased height of contraction at the end of a "staircase" is associated with a slower development and subsidence of the contractile process. Thus the whole effect is nothing more than a preliminary stage of fatigue and it is not surprising that the $H^{\cdot}$ ion concentration of the perfusing fluid should make very little difference to its onset except in so far as it may hasten or retard fatigue.

On the other hand in the heart the increased height of a contraction occurring in the supernormal phase of recovery is certainly not due to fatigue. Fig. 6 shows that there is no appreciable difference in the duration of the first and second of a pair of contractions, though the height of the second may be very much greater than that of the first. This is confirmed by records of the contractions made on a rapidly moving drum. Fig. 7 shows such a record from a heart which had been perfused for half an hour with a fluid of P_H 6·4. The time marker signals every second. The second contraction is 130 p.c. as high as the first, but the duration of the two contractions is exactly the same.

Thus Fröhlich's explanation will not cover the case of the supernormal contractions of cardiac muscle, though it is evidently true for the "Treppe" in skeletal muscle. In fact the two effects are essentially different, as indeed their time relations would lead one to suppose.

II. THE NATURE OF THE SUPERNORMAL PHASE.

It has been shown that in a tissue which is in equilibrium with an acid perfusing fluid the three main functions of excitability, conductivity and contraction are all increased above their normal resting value at a certain stage in the recovery from a previous disturbance. The discussion which follows is concerned mainly with the increased excitability because this function is more easily investigated than conduction or contraction. Also the experimental results are more easily interpreted because the excitation is an immediate consequence of the external stimulus, whereas many processes may intervene between the stimulus and the contractile response.

(a) *Factors accounting for the increased excitability.*

Within recent years the mechanism of electrical excitation has been worked out by Nernst, Lapicque, Lucas, Hill and others, and it is generally agreed that the necessary condition for excitation is that the current should produce a certain heaping up of ions at some membrane in the tissue within a certain time. With brief currents such as the break shocks of a coreless induction coil the time limit need not be considered, since it will always outlast the stimulating current. Thus the success or failure of such a current will depend on two factors (*a*) the ease with which the ions can be moved up to the membrane, and (*b*) the concentration which must be produced at the membrane. The factor (*a*) will depend on the mobility of the ions concerned; the concentration brought about by the current will tend to dissipate itself by backward diffusion, and if the mobility of the ions is increased this escape by backward diffusion will outweigh the greater rapidity with which the ions will move up to the membrane under the influence of the electric field.

Keith Lucas (10) showed that it is possible to estimate the share of either of these factors in causing a given alteration of excitability by mapping out the curve relating current duration to current strength required to excite. If the shape of the curve is not altered and the only effect of the change has been to increase by a constant percentage the current strength corresponding to each duration, then the change must be due entirely to an increase in the concentration of ions required at the membrane and the mobility of the ions has not been affected. If the shape of the curve changes, then the mobility of the ions must have altered, and the extent of this alteration may be calculated from the constants of the curve according to Hill's equation (11).

Thus at the outset we may enquire whether the increased excitability during the supernormal phase is due to a decrease in the mobility of the effective ions or to a decrease in the concentration required at the membrane. To do this we must map out the strength-duration curves for currents sent into the resting nerve and for currents sent in during the supernormal phase of recovery. Fortunately the excitability of the nerve remains approximately constant for about ·005 sec. during the height of the supernormal phase, and the complete strength-duration curve can be mapped out without using durations greater than this.

Fig. 9 shows the result of an experiment carried out in this way. The nerve was perfused for two hours with a fluid of P_H 6, and the recovery curve was then determined. At an interval of ·0216 sec. after the first stimulus the excitability had risen to 115 p.c. of its resting value, and it remained at this level for ·017 sec. The strength-duration curve was then determined (*a*) for the resting nerve, and (*b*) with currents which began

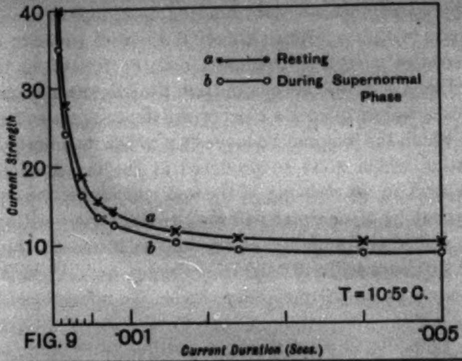


FIG. 9

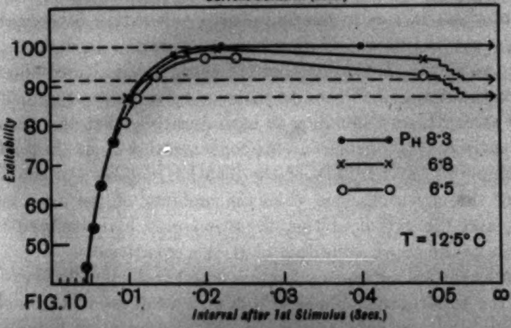


FIG. 10

Fig. 9. Relation between strength and duration of current required to excite (*a*) during rest and (*b*) during supernormal phase of recovery.

Fig. 10. Recovery of excitability in different fluids. Not corrected for change of threshold.

·024 sec. after a single maximal stimulus. These currents would fall on tissue which was at the height of the supernormal phase. The two strength-duration curves are shown in the figure: the upper curve (*a*) is that for the resting nerve and the actual measurements are shown by the small dots on the curve, the minimal current required to excite being given the value 10; the lower curve (*b*) is that determined during the supernormal phase, and the minimal current has the corresponding value of 8·7, as the excitability was increased to 115 p.c. The crosses on the upper curve represent the current-strengths of the lower curve multiplied by $\frac{115}{100}$. They agree so closely with the points determined for the resting nerve that there can be no doubt that the shape of the two curves is identical.

Thus the increased excitability during the supernormal phase must be due to a decrease in the concentration of ions necessary for excitation to occur and not to any alteration in the mobility of the ions concerned.

(*b*) *The effect of different concentrations of $H^{\cdot}$ ions.*

The most striking feature of all the foregoing experiments has been the appearance of the supernormal phase when the perfusion fluid is on the acid side of neutrality and its absence when the fluid is on the alkaline side. The curves in Figs. 2 and 3 do not suggest any explanation of this effect because the curve for each fluid is drawn on the assumption that the resting excitability in that fluid has the value 100. This is legitimate enough as a means of showing the general effect of different fluids on the form of the recovery curve, but it misses one of the most important features of the experiment. The resting excitability has a different value for each fluid, and if we allow these differences to appear instead of reducing all the curves to the same threshold line the changes in the form of the recovery curve become much more significant. Fig. 10 is constructed on these lines from an experiment in which the nerve was perfused with fluids of P_H 8·3, 6·8 and 6·5. The resting excitability in the fluid of P_H 8·3 is taken as 100 and the recovery curve in this fluid is shown gradually rising to this level and remaining there. The actual determinations are marked by small dots on the curve. After half an hour in a fluid of P_H 6·8 the excitability had fallen to 91·5 p.c. of its former value. Consequently the normal is now 91·5 instead of 100, and the recovery curve is constructed on this basis, the small crosses showing the actual measurements. In the fluid of P_H 6·5 the excitability fell from 91·5 to 87 p.c. and the recovery curve is marked by the small circles.

The two "acid" curves show the usual supernormal period and the "alkaline" curve does not, but the chief point of interest lies in the fact that in the earlier stages of recovery all three curves are absolutely continuous, and that they are very nearly continuous up to the height of the supernormal phase of recovery. Thus at ·022 sec. after the first impulse the excitability in P_H 8·3 is 99·5, that in P_H 6·8 is 99 and that in P_H 6·5 is 97. At longer intervals the excitability in the acid fluids falls off and eventually reaches the resting values of 91·5 for P_H 6·8 and 87 for P_H 6·5. Evidently in fluids of different P_H the maximum excitability at the height of the supernormal phase is much more nearly constant than the normal excitability after recovery is complete.

The same result was found in all the nerves examined. The maximum excitability in a fluid on the acid side of neutrality was always slightly less than the excitability in an alkaline fluid, but the difference was usually not greater than 5–6 p.c. whereas the difference between the resting excitabilities was often as great as 20 p.c. The result was quite independent of the order in which the different fluids were perfused, though three experiments had to be discarded because the nerve was obviously undergoing a progressive change and the thresholds were not constant.

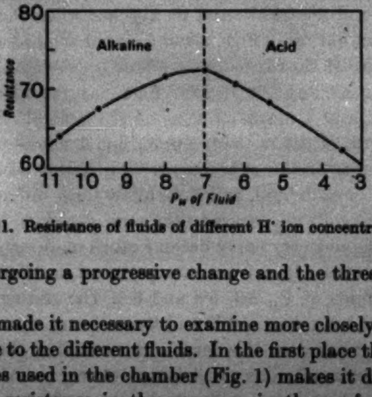


Fig. 11. Resistance of fluids of different H˙ ion concentration.

This result made it necessary to examine more closely the changes in excitability due to the different fluids. In the first place the arrangement of the electrodes used in the chamber (Fig. 1) makes it difficult to allow for changes in resistance in the nerve or in the perfusing fluid. The current is carried partly by the nerve and partly by the fluid surrounding it, and therefore an increase in the resistance of the fluid will cause a larger proportion of the current to pass through the nerve, and *vice versa*. To gain some idea of the magnitude of this effect the resistance of the different fluids was measured: it was found that the resistance was greatest at the neutral point (P_H 7), that it decreased when the fluid was made more acid or more alkaline and that the decrease was more rapid for increasing acidity than for increasing alkalinity. The values of the resistance in arbitrary units are shown in Fig. 11, and it will be seen that a fluid of P_H 6 has a resistance of 70·25 and one of P_H 5 a resistance of 67·5 as against the values of 71·5 for P_H 8 and 69·5 for P_H 9. This result is only to be expected in view of the fact that the H˙ and OH˙ ions are more mobile than the other ions present in the fluid and that the H˙ ion is more mobile than the OH˙. The differences are small, but they are of such a nature that a nerve in an acid fluid will appear to have a slightly lower excitability than one in an alkaline. The resistance of the nerve shows no appreciable change after perfusion with the different fluids; it was measured with a weak alternating current led in by non-polarisable electrodes.

To overcome the effect of these small changes in resistance the experiment was conducted in a slightly different way. The nerve was placed on platinum electrodes immersed in the perfusing fluid and the second stimulus was a brief constant current of ·0008 second duration derived from a low resistance potentiometer wire in circuit with a four volt accumulator. With this arrangement the potential difference between the two electrodes must remain very nearly constant in spite of large changes in the resistance of the perfusing fluid, since these changes will have practically no effect on the fall of potential in the potentiometer.

Figs. 12 and 13 show two sets of curves obtained by this method. In Fig. 12 the maximum excitability in an acid fluid is 99 p.c. of the resting value in a neutral fluid. In Fig. 13 the agreement is not so close and the supernormal excitability in P_H 5·5 is only 95 p.c. Both figures show that the resting excitability in an alkaline fluid is less than it is in a fluid nearer the neutral point. This is only true within certain limits, for a strongly alkaline fluid (P_H 11) usually causes a great initial rise of excitability followed by a sudden fall which heralds the complete failure of all stimuli. This rise is probably due to some secondary effect on the fibre, for as long as the P_H does not exceed 9·5 the excitability is generally less than it is at neutrality.

Thus there is still a slight discrepancy between the supernormal excitability in an acid fluid and the normal in a neutral fluid even when changes in resistance are taken into account. However there is another factor which has not yet been considered and this is the decrease in the mobility of the kations when the H˙ ion concentration of the fluid is reduced. As stimulation takes effect at the kathode, the mobility of the kations will be an important factor in deciding whether a given current can establish the required concentration at the membrane against the opposing forces of diffusion. In a fluid on the acid side of neutrality there will be large numbers of the very mobile H˙ ion, whereas in an alkaline fluid the slower Na˙ ions will take a larger share in the work. For this reason the current required to produce a given concentration of kations will be weaker when the fluid is alkaline than when it is acid.

The magnitude of this effect can be tested by constructing the curves

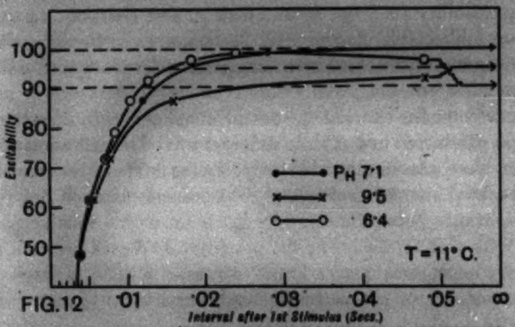


FIG. 12

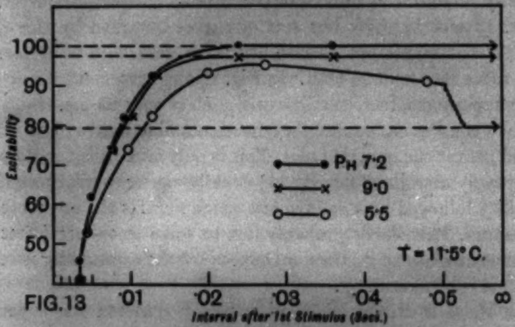


FIG. 13

Figs. 12 and 13. Recovery of excitability in different fluids. Not corrected for change of threshold.

relating the strength and duration of the stimulating current in fluids of different P_H (see p. 17), since any change in the time factor of the curve must be due to an altered rate of diffusion. It was found that a change from acid to alkali did produce an appreciable shift in the curve. Fig. 14 shows two curves from a nerve perfused with fluids of P_H 9·6 and P_H 5·5. The current strength at infinite duration is given the value 10 in either case, though the threshold was in reality 36 p.c. higher in the acid fluid than in the alkaline. It will be seen that the current strength in P_H 9·6 rises at greater durations than that in P_H 5·5, and the chronaxie (*i.e.* the duration corresponding to twice the strength at infinite duration) is ·00045 sec. in the former and ·00035 sec. in the latter. A change of the same order was found in the two other preparations which were examined.

It is impossible to insist on the exact numerical values found in this experiment, because the probable error is large, but it is clear that the altered excitability in fluids of different P_H must depend on some other factor besides an alteration in the required concentration of ions, and the direction in which the curve is shifted shows that in an acid fluid there is an increased tendency for differences of concentration to be equalised by diffusion. This means that a part of the increased strength of current required to excite will be needed to counteract the greater rapidity of diffusion of the $H^{\cdot}$ ions.

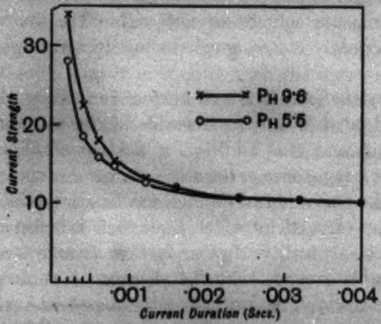


Fig. 14. Relation between strength and duration of current required to excite resting nerve in fluids of different $H^{\cdot}$ ion concentration.

From Hill's equation it is possible to calculate the relative importance of the change in the rate of diffusion and the change in concentration required at the membrane. In this particular experiment it appears that about one-quarter of the whole change in threshold is due to the altered rate of diffusion, and three-quarters to a change in the required concentration. Now it was found that the resting excitability in an acid fluid might be 21 p.c. less than that in a neutral fluid, whereas the supernormal excitability in the acid fluid was only 5 p.c. less (Fig. 13).

It has been shown that the rate of diffusion of the ions is the same in the supernormal phase of recovery as in the resting state (Fig. 9) and therefore if we consider only the concentration of ions necessary to excite it is clear that, in the supernormal phase, this may be equal to or even less than the concentration required in a neutral fluid.

In fact if instead of the strengths of current needed to excite, we take the concentration of ions which must be brought about in the tissue, we have the result that for the resting nerve the concentration is least when the fluid is neutral and that it becomes greater when the fluid is made more acid or more alkaline, but that during the supernormal phase of recovery in an acid fluid the necessary concentration is not greater than that required at neutrality.

It would be idle to pretend that an exact agreement can be proved from the experiments submitted, but within the limits of error the agreement is certainly close enough to justify some speculation as to its cause.

Suggested explanation. It is well known from the work of Hardy (12) that a colloidal particle is most unstable in the neighbourhood of its iso-electric point, and that addition of acid or alkali makes it more stable by giving it a positive or negative charge. Let us assume that the membrane in the nerve fibre at which the concentration of ions takes effect is made up of particles which have their iso-electric point in the neighbourhood of neutrality. Let us further assume (and here we are on more debatable ground) that the passage of an impulse with its corresponding electric variation leaves the membrane with a negative charge which becomes smaller and smaller as the tissue recovers. This would mean that as recovery advanced the tissue would become more and more easy to excite, because the particles forming the membrane would become more and more unstable as their negative charge was lost. In a neutral fluid the particles would end up with no charge at all and in this condition the tissue would be most easily excited; in an alkaline fluid the particles would retain a small negative charge and consequently the excitability would never rise to the maximum value, on the other hand in a fluid on the acid side of the iso-electric point the particles on losing their negative charge would pass through a phase in which they had no charge at all and they would then take on a small positive charge from the $H^{\cdot}$ ions in the fluid. Thus the nerve recovering in an acid fluid would pass through a phase in which its excitability was equal to the maximum and would then become less excitable again as the membrane became positively charged.

An explanation on these lines would account for the appearance of the supernormal phase only when the nerve is in equilibrium with an acid fluid and for the close agreement between the excitability in the supernormal phase and the resting excitability in a neutral fluid. It would also account for the phenomena of hysteresis mentioned on p. 7, since Mines has shown that the charge conferred on a membrane by ions of different sign behaves in the same way(13).

Nevertheless it cannot be claimed that the hypothesis as it stands is free from objection. It is a far cry from a protein particle to a membrane which is probably the seat of an electrical double layer even when it is in equilibrium with a neutral fluid; again, the most vital assumption of the theory, that the excitable membrane becomes negatively charged during the passage of an impulse, is by no means certain. It is true that the passage of an impulse involves an electric variation in which the active part of the nerve becomes negative to surrounding parts as judged by electrodes placed in contact with the nerve trunk, but most of the evidence goes to show that this variation is due to the actual passage of a current in the surrounding fluid from the resting to the active part of the nerve, and it does not follow that such a current would produce a negative charge on the protein particles at the point where it re-entered the nerve. Another objection is that it is by no means certain how far the recovery of excitability after an impulse runs parallel with the subsidence of the electric response, and there are some grounds for denying any relation between the two(14).

It would however be premature to discuss these and other difficulties until further evidence is collected. The hypothesis must be regarded as nothing more than an indication for further work, and I am at present engaged on a series of experiments which may give more definite grounds for accepting or rejecting it.

III. Theories of acid production.

Although the hypothesis put forward cannot be insisted on at present, the facts on which it is based do enable us to test one particular development of the theory which regards the activity of an excitable tissue as due to the production of acid. That acid is produced is not disputed, but the point at issue is whether the production of acid precedes or follows activity. In the case of nerve there is little doubt that if acid is produced at all (and Tashiro's(15) results make this highly probable), the production cannot be a sudden event occurring at the same moment as the change which constitutes the impulse. As Bernstein(16) and

Brünings(17) have pointed out, the fact that the electric variation in nerve is not accompanied by any appreciable change of temperature makes it necessary to suppose that the current is caused not by any simultaneous chemical change, but by the liberation of free energy due to the existence of inequalities in the concentration of different ions in different regions in the fibre. In fact the nerve behaves like a concentration cell, and the production of a current without any appreciable change of temperature is comparable to the performance of work by a perfect gas expanding isothermally. This conception is greatly strengthened by the discovery by Bernstein and Tschermak(18) that the electric organ of the Torpedo shows a distinct cooling when the current it produces is led through an external circuit. The current will develop heat in passing through this circuit, but the amount of heat evolved must be exactly balanced by the cooling of the organ, since there is no change of temperature at all when the current has to travel in the fluid immediately surrounding the organ instead of passing through an external circuit. Thus the work done is derived ultimately from the warmth of the surroundings, though it involves a decrease in the free energy of the concentration cell.

After the impulse has passed, the differences of concentration which existed previously must be restored, and this process will ultimately involve a chemical change which may be associated with the production of acid. But this change may be spread over a long period, just as the recovery heat production of a muscle may continue for two or three minutes after the contraction has ceased.

Thus in nerve it seems highly probable that the passage of an impulse involves a change of physical rather than of chemical energy, and that it cannot be due to any sudden production of acid in the fibre[1].

In the case of muscle the reverse is generally assumed to be true and it is supposed that the contractile process is the result of a sudden production of acid in contact with the sensitive fibril mechanism. The particular form of this theory which concerns us at present is that stated by Mines(19). He says "Our hypothesis emerges in the following terms. The propagated disturbance in muscle culminates in the liberation of acid in certain localised regions of the muscle fibre. The subsequent shortening is due to the action on some colloidal system of these local concentrations of acid: the local relatively high concentrations rapidly disappear by diffusion and the general rise in $H^{\cdot}$ ion concentration (a rise which is gradually counteracted by the oxidative removal of the acid) is responsible for the more lasting effects of excitation on excitability and tone." On these lines he explains the summation of contractions and the "Treppe" of skeletal muscle, and the increased height of the second contraction in cardiac muscle. In regard to the heart he shows first of all that the force of contraction is greatest when the heart is in equilibrium with a fluid of $H^{\cdot}$ ion concentration in the neighbourhood of neutrality. He considers that the tissue of the resting heart will be on the alkaline side of this optimal value, but that the occurrence of a contraction will cause a liberation of acid which will temporarily increase the general $H^{\cdot}$ ion concentration of the tissue. As the increased $H^{\cdot}$ ion concentration is subsiding it must pass through its optimal value (near neutrality) before it returns to its resting value on the alkaline side of neutrality. Consequently a stimulus occurring at the moment when the concentration is optimal will produce a larger contraction than that set up in the resting muscle.

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[1] Another argument in favour of this is the difficulty of explaining the electric variation as due to the sudden production of acid (see Lillie, *Amer. Journ. of Physiol.*, 37. p. 354. 1915).

It is difficult to reconcile this attractive hypothesis with the behaviour of the heart described on pp. 10–13. There can be little doubt that the $H^{\cdot}$ ion concentration of the perfusing fluid bears some relation to that of the tissue when at rest, and little doubt that the force of contraction is greatest when the tissue is at or near neutrality. But on Mines's hypothesis the second contraction should be larger than the first only when the tissue passes through the optimal concentration on its way back to the resting value; this should occur when the resting tissue is alkaline but not when it is acid, for then the optimal concentration will never be reached. Thus we should expect to find the supernormal phase of recovery present when the tissue is in equilibrium with an alkaline perfusing fluid and absent when the fluid is acid. Actually we find the exact reverse of this; the supernormal phase is always present in an acid fluid and never in an alkaline. Indeed Mines's hypothesis would be in exact agreement with the limited range of facts here presented if we read $OH^{\cdot}$ ion for $H^{\cdot}$ ion and suppose that the contraction is due to a liberation of alkali instead of acid.

This proposal would be in conflict with so many well-known phenomena that it need not be seriously considered. As an alternative to Mines's hypothesis I can only offer the suggestion already put forward to account for the supernormal phase of excitability, namely that the force of contraction depends, like the excitability, on the initial degree of instability of some surface in the muscle, that this instability is greatest at the iso-electric point and that activity causes the surface to take on a negative charge whereas an acid perfusing fluid gives it a small positive charge. Applied to explain the recovery of contractile power this hypothesis has all the defects which it has in explaining the recovery of excitability, and probably many others as well, but a discussion of these points must be left to a future occasion.

It might appear unnecessary to suppose that the increased contraction was due to any direct effect on the contractile mechanism in each element of the muscle, since it could be explained by an increase of conductivity which would bring into play parts of the muscle which were not accessible to a single stimulus. This explanation has been invoked already by Lucas and Adrian (20) to account for the increased size of the second electric response in a muscle stimulated indirectly. Here there is some justification for it because the fibres of a skeletal muscle may not all be brought into play by a single stimulus, but it is very doubtful if the same explanation could apply in the heart where the whole of the musculature responds together if it responds at all. The point need not be considered at length as it was fully dealt with by Mines in his paper.

The foregoing arguments are not intended to dispute the view that the contraction of a muscle may be due to the liberation of acid, but they do throw considerable doubt on the extension of this view to cover the favouring effects of previous activity in cardiac muscle.

IV. THE MECHANISM OF THE SUMMATION OF IMPULSES.

In the course of this work certain facts have emerged which have an important bearing on the theory of nervous summation.

Lucas and I showed that the conditions necessary for summation in the peripheral nerve were as follows: in the first place the nerve or some part of it must conduct with a decrement so that a single impulse cannot pass through, secondly the nerve must exhibit a supernormal phase in recovery, and thirdly it must be stimulated by a series of impulses so timed that each falls in the supernormal period following its predecessor. Under these conditions a single impulse will have no effect on the tissue beyond the region of decrement but a series of impulses will succeed in passing through. We supposed that the same mechanism would account for the summation of impulses in a reflex arc, the region of decrement being the junctional or synaptic region between one neurone and another. To account for the great variety of response in the central nervous system we must add that the degree of decrement existing at the synapse varies from one moment to another according to the state of fatigue of the arc, the general condition of the body, etc.

Now the facts brought forward in this paper show that no summation of this kind will be possible unless that part of the nervous arc which conducts with a decrement is in equilibrium with a surrounding fluid on the acid side of neutrality. In terms of the hypothesis on p. 24 we should say that no summation is possible unless the membranes in this part are positively charged. There is no direct proof that this condition obtains in the central nervous system, but if we consider how rapidly it is fatigued and how much it depends on its oxygen supply, it seems highly probable that the imperfect conduction at a synapse will vary with the degree of acidity of the tissue and the existence of the decrement may be due to the tissue being in equilibrium with an $H^{\cdot}$ ion concentration greater than the iso-electric value. Thus the two conditions of a decrement in conduction and a supernormal phase may be both fulfilled by the same factor, and this factor is likely to change from moment to moment according to the state of fatigue of the arc.

Evidently the nervous paths in the grey matter are much more sensitive to changes in acidity than are the peripheral nerves. It is as important for the organism that the peripheral nerves should never vary in conductivity as it is that conduction in the central nervous system should change from one minute to the next. Thus the peripheral nerves in a frog conduct without a decrement between such wide limits as P_H 10 and P_H 4, whereas the respiratory centre in a mammal responds at once to a minute change in the P_H of the fluid surrounding it.

CONCLUSIONS.

1. A nerve which is recovering from the passage of a previous impulse usually shows a supernormal phase of recovery in which its excitability and conductivity are greater than in the resting state. The supernormal phase is not always present and its extent is variable.

2. The supernormal phase depends on the $H^{\cdot}$ ion content of the fluid with which the nerve is in equilibrium; if the fluid is on the acid side of P_H 7 the supernormal phase is well marked and it increases with increasing acidity; on the alkaline side of P_H 7·4 the supernormal phase is absent. The above holds good for the recovery of conductivity as well as that of excitability.

3. In resting cardiac muscle the second of two contractions may be greater than that of the first. This increase in contractile power above its resting value is part of the supernormal phase of recovery. It is

associated with an increased excitability and it is present when the heart is perfused with an acid fluid and absent with an alkaline.

4. The staircase effect in skeletal muscle is an entirely different phenomenon. It depends, as Fröhlich has shown, on the increased duration of the contractile process in the early stages of fatigue. It is not affected by the $H^{\cdot}$ ion content of the perfusing fluid.

5. An analysis of the recovery of excitability in nerves shows that the maximum supernormal excitability in an acid fluid is very slightly less than the excitability in a neutral fluid, whereas the resting excitability is very much less.

6. If instead of the current required to excite we consider the concentration of ions which must be brought about in the tissue by this current, it appears that the minimal concentration required to excite during the supernormal period in an acid fluid is equal to the minimal concentration required in a neutral fluid, although the resting value in an acid fluid is very much greater.

7. To account for these facts it is suggested that the excitability, conductivity and contractile power are greatest when the colloidal particles which form the membranes of the tissue are initially uncharged, that a fluid on the acid side of the iso-electric point produces a small positive charge and that activity produces a negative charge. As this negative charge subsides the instability of the tissue increases and reaches its highest value when the surface is iso-electric. If the tissue is in an acid fluid the surface must pass through the iso-electric state and end up with a small positive charge, whereas in an alkaline fluid the iso-electric state is never reached and therefore the excitability never rises above its resting value. The complete discussion of this hypothesis is postponed until more data are available.

8. It is difficult to accept Mines's theory that the increased second contraction in heart muscle is due to the production of acid by the first contraction and the consequent increase in the $H^{\cdot}$ ion concentration of the tissue to its optimal value. If this were so the supernormal phase should be present in an alkaline fluid and absent in an acid, whereas the reverse is the case.

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EXPERIMENTS ON THE REGULATION OF THE BLOOD'S ALKALINITY. I.

BY H. W. DAVIES, M.B., B.S., J. B. S. HALDANE, M.A., *Fellow of New College, Oxford* AND E. L. KENNAWAY, D.M., D.Sc.

THE following experiments were carried out in Dr J. S. Haldane's laboratory, Cherwell, Oxford, and our thanks are due to him for suggesting many of the experiments, and for constant advice and criticism. The majority of the urine analyses were carried out by E. L. K. at the Bland Sutton Institute of Pathology, Middlesex Hospital. We are indebted to Mr Ralph Segnit for the drawings of Figures 1, 2 and 3.

Methods. All gas mixtures were analysed with the small type of Haldane's apparatus. Alveolar CO_2 was estimated by Haldane and Priestley's method, each determination being the mean of an inspiratory and expiratory sample. The volumes of CO_2 taken up by 1 c.c. of defibrinated blood were estimated with Brodie's modification of the Barcroft-Haldane apparatus by the method described by Christiansen, Douglas and Haldane(1). This apparatus was also used for estimating the bicarbonates of the urine by the volume of CO_2 given off on adding acid. The excretion of acid and alkali was also arrived at by titrating the urine to P_H 7·4 as follows: a box, with the back replaced by white paper, is prepared to hold three test tubes of equal calibre. Two of these placed at the sides contain 10 c.c. of a phosphate mixture of P_H 7·4 (19 vols. M/15 KH_2PO_4 and 81 vols. M/15 $Na_2HPO_4 2H_2O$) to which is added 12 drops of neutral red solution (0·05 p.c. in alcohol). The acid urines were titrated with 0·1 *N* NaOH. If the alkaline urines were titrated directly the results were enormously too low (sometimes 1/15th of the true value) owing to retention of CO_2, which comes off very slowly. They were therefore titrated as follows: in a wide test tube is placed—

(*a*) 5 c.c. of urine.

(*b*) 0·1 *N* H_2SO_4 in amount sufficient to produce a strongly acid reaction to neutral red, and water if necessary to make up to 15 c.c. If the urine so diluted shows any appreciable colour in a test tube water is added and the amount of indicator increased proportionally.

(*c*) 24 drops of the neutral red solution.

A strong current of air is then drawn through for 10 minutes (5 minutes were actually sufficient), more acid being added if the reaction does not remain distinctly acid. Liquid paraffin is used to prevent frothing, as caprylic alcohol extracts neutral red near the neutral point. The excess of acid is then titrated with 0·1 *N* NaOH; the more alkaline urines neutralised 12 or 13 c.c. of the 15 c.c. acid added. Towards the close of the titration 10 c.c. of the fluid is poured from the titration flask into the middle test tube in the box between each addition of alkali, and is returned to the flask after comparison with the phosphate solution, and so on until the reaction required is reached. The end-point is extremely sharp.

The weakly alkaline urines were titrated as above except that ·02 *N* solutions were used. The gas method and the titration method agreed within 5 p.c. or less for high concentrations, but somewhat less accurately for low concentrations, probably owing to the presence of Na_2HPO_4. Hence in the more alkaline urines nearly all the alkali was excreted as bicarbonate.

Total nitrogen was estimated by Kjeldahl's method, and the ammonia by the air-current method. The presence of aceto-acetic acid was determined by Rothera's test, but was never estimated quantitatively. The concentration probably never reached ·1 p.c.

CO_2-carrying capacity of the blood. As a preliminary the CO_2 dissociation curve of the normal blood of H. W. D. was determined. The results are given in Fig. 1. In this figure the combined CO_2 only is given; the dissolved CO_2 is calculated, using Bohr's (2) value of ·511 for the solubility of CO_2 in blood, and the value thus obtained is deducted from the total volume given off. The exact figures obtained are given in Table I.

To obtain the points for high pressures of CO_2 we used mixtures of CO_2 and O_2, as owing to the tendency of CO_2 to dissociate oxyhæmoglobin it appeared desirable to guard against any possibility of incomplete oxidation of the hæmoglobin. Some experiments which are not yet complete indicate that at 400 mm. pressure of CO_2 reduced blood takes up only about two to three volumes p. c. more CO_2 than oxygenated blood. It was also considered possible that the high pressures of CO_2 might cause some irreversible changes in proteins resulting in an increased CO_2 capacity. To settle this question, a sample of blood was saturated at a pressure of 497 mm. of CO_2. A portion of the sample was then placed in the blood gas-apparatus and the volume per cent. of CO_2 absorbed was found to be 121·1. The remainder of the sample was then resaturated at 48·2 mm. pressure of CO_2, when the volume p.c. of CO_2

absorbed (after correction for the additional time spent in the bath, *vide* Christiansen, Douglas, and Haldane's paper) was found to be 53·6, a normal result. Hence any important changes of the proteins must be reversible.

The results obtained agree with those of Christiansen, Douglas, and Haldane(1) and with Parsons'(3) experimental results, being on the whole very slightly lower than the former, but the volume of CO_2 taken up was invariably a good deal higher than the value found by Joffe and Poulton(4). The high values did not agree with those calculated from Parsons'(3) equation. Thus the blood of J.S.H. took up 98·6 volumes of combined CO_2 at a pressure of 428 mm. According to

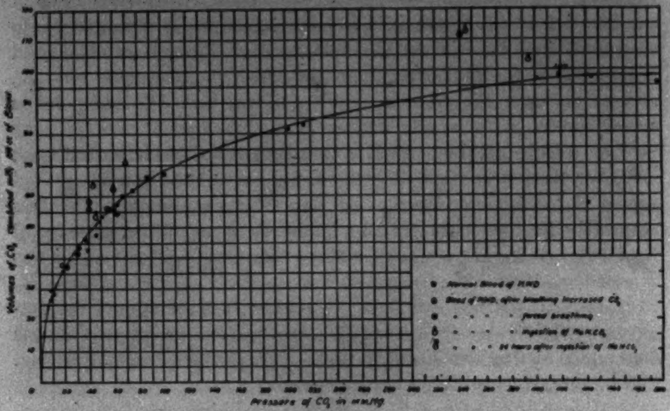

Fig. 1

TABLE I. CO_2-carrying capacity of normal blood of H. W. D.

mm. pressure of CO_2	Vols. of CO_2 taken up by 100 vols. of blood	Vols. of combined CO_2	mm. pressure of CO_2	Vols. of CO_2 taken up by 100 vols. of blood	Vols. of combined CO_2
8·3	26·9	26·3	64·9	61·8	57·4
9·02	30·2	29·6	68·5	64·6	60·0
11·2	29·4	28·7	76·7	67·2	62·0
19·3	36·8	35·5	89·9	71·7	65·7
29·8	43·4	41·4	102·9	73·4	66·5
38·9	49·0	46·4	202	95·4	81·8
39·0	48·1	45·5	215	97·0	82·6
39·1	45·7	43·1	411	125·0	97·3
46·6	50·4	47·3	455	128·2	97·6
51·3	56·6	53·2	497	129·1	95·7

Parsons' equation it should only take up 90·5 volumes of CO_2 at this pressure, a pressure of 796 mm. being required before 98·6 volumes are taken up. This fact shows that the proteins (of which no doubt hæmoglobin is the most important) cannot quite correctly be taken as equivalent to a single weak acid. If we supposed, as is *a priori* probable, that the different acid side chains of the hæmoglobin molecule have somewhat different dissociation constants, this would account for the divergence of our results from those expected on Parsons' comparatively simple theory.

Since the CO_2 taken up in combination by the blood does not vary appreciably (only between 95·4 and 97·5 vols. p.c.) between CO_2 pressures of 411 and 496 mm. it is clear that at these pressures almost all the available base is combined with CO_2. If then we saturate blood with CO_2 at a high pressure we shall obtain a true measure of the available base or "alkaline reserve." At lower pressures a certain amount of this base is combined with protein, and as Parsons' equation is not quite exact, it is impossible to determine exactly how much. If on the other hand we find that 100 c.c. of abnormal blood take up 16 vols. more of CO_2 than 100 c.c. of normal blood we know that they contain 16 N/2240 or N/140 base above the normal content. In the living subject some of this would be combined with protein.

Effects of breathing increased percentages of CO_2. These experiments were carried out in a large air-tight respiration chamber of about 260 cubic feet capacity. CO_2 was passed in through a meter in amounts sufficient to give approximately the required percentage (6 p.c.). The subject then entered the chamber having previously passed urine. Samples of air were taken for analysis immediately after entering, and just before leaving, the chamber. Three separate experiments were performed on H. W. D. the duration of each being about 2 hours. After entering the chamber the respirations gradually increased, in a few seconds reaching a maximum depth, with frequency varying between 22 and 30, and continued so throughout. No marked rise of pulse rate was observed. A second sample of urine was collected about half an hour after entering the chamber, and a third just before the conclusion of the experiment. The blood sample was also taken just prior to leaving the chamber. In the first experiment the percentage of CO_2 breathed at the commencement was 5·22, rising to 5·59 just before leaving the chamber. In this experiment no samples of urine were taken. About eight minutes after entering the chamber a slight headache came on which lasted throughout the experiment. In the two subsequent experiments the CO_2 percentage varied between 6·01 and 6·45. No headache occurred in the chamber, but headache of moderate severity, with sudden onset and lasting several hours, was observed immediately after emerging. In none of these three experiments was there any appreciable alteration of the CO_2 dissociation curve of the blood (*vide* Fig. 1). There was possibly some slight increase in the CO_2-capacity of the blood but the differences from the normal hardly exceed the errors of experiment. This result contrasts with the previous results of Yandell Henderson and Haggard(5) in their experiments on animals. Probably the percentage of CO_2 breathed in our experiments was insufficient (they used over 10 p.c.), or the duration of the experiments was too short, to allow of the redistribution of alkali in the body as discovered by these observers.

Table II shows the results of urine analyses in the last two experiments, the main features of which are:

(*a*) Diuresis—the volume per hour increasing almost fourfold.

(*b*) Increased excretion of acid—the faintly alkaline urine of 26/9/19 becoming acid, and the strongly alkaline urine of 3/10/19 becoming almost neutral. (In these titrations no attempt was made to remove CO_2 as in the method described above.)

TABLE II. Analyses of urines. Breathing CO_2.

					To bring urine to P_H 7·4 requires c.c. 0·1 N			
			Volume c.c.		Per 100 c.c. urine		Per hour	
Date	Time	Time mins.	Total	Per hour	Acid	Alkali	Acid	Alkali
26/9/19	9 a.m.— 1.50 p.m.	290	305	63	1·60	—	1·01	—
	1.50 p.m.— 2.25 „	35	155	266	—	1·10	—	2·92
	2.25 „ — 3.50 „	85	320	226	—	5·60	—	12·70
3/10/19	11 a.m.—12.30 p.m.	90	185	124	14·90	—	18·40	—
	12.30 p.m.— 1 „	30	130	260	6·00	—	15·60	—
	1 „ — 2.45 „	105	778	445	1·00	—	4·40	—

	NH_3 c.c. 0·1 N		Total N Mg			
	Per cent.	Per hour	Per cent.	Per hour	NH_3 N p.c. of Total N	Remarks
26/9/19	9·66	6·08	·613	387	2·20	H. W. D. entered chamber at 1.54 p.m. CO_2 5·35 p.c.
	5·20	13·80	274	728	2·65	2.30 added 4 cu. ft. CO_2 2.40 CO_2 6·17 p.c
	5·30	12·00	195	440	3·80	3.50 CO_2 6·17 p.c.
3/10/19	3·14	3·88	554	685	0·79	H. W. D. entered chamber at 12.27
	2·80	7·28	270	702	1·45	12.30 CO_2 6·01 p.c.
	1·88	8·34	138	613	1·90	2·45 CO_2 6·47 p.c.

Rothera aceto-acetic acid—nil throughout.

(*c*) The ammonia excretion per hour was doubled in both experiments.

The increase of ammonia is analogous to that found by **Walter** (6) in dogs to which HCl had been given; and to that of diabetic acidosis. It is presumably due to the action of increased acid of the plasma in shielding ammonia from conversion into urea by the liver. The increased output of ammonia per hour was of the same order of magnitude as the increase of acid or decrease of alkali excreted per hour in the urine, and in one experiment was equivalent to about half the increased acidity and in the other to about one-third. The liver was therefore from half to one-third as efficient as the kidneys in compensating the acidosis.

Forced breathing experiments. It has previously been shown by **Henderson** and **Haggard** (5) that excessive pulmonary ventilation in animals, whether induced by ether, by mechanical means, or by shock, causes a disturbance of the CO_2-carrying capacity of the blood. In addition **Leathes** (7) has shown that in the human subject a moderate degree of forced breathing can be maintained for considerable periods and that under such circumstances there is increased excretion of alkali by the kidneys.

We have endeavoured to extend the observations of **Henderson** and **Haggard** to man, but find that with the amount of forced breathing voluntarily possible in the human subject, there is no alteration of the CO_2-carrying capacity of the blood as evidenced by alteration of the CO_2 dissociation curve (*vide* Fig. 1). Three experiments were performed. In the first two the duration of the forced breathing was one hour. The first sample of urine was taken 15 minutes after commencing forced breathing, and the second, together with the sample of blood, at the conclusion. Considerable discomfort was felt by the subject, the main symptoms observed being numbness and tingling of the extremities, fibrillary twitching of the orbicularis palpebrarum, and slight Rombergism. These symptoms were mostly relieved by the inhalation of a few breaths of pure oxygen. The results of the urine analyses of the first two experiments are shown in Table III.

Details of the later experiment are given in the protocol. Outstanding features are:

(*a*) Diuresis.

(*b*) Marked increase in the alkalinity of the urine (*vide* protocol).

(*c*) Lessened excretion, and, on 4/10/19, complete absence of ammonia in the urine.

(*d*) The excretion in the first two experiments of doubtful traces of acetone bodies, and in the later experiment of a moderate amount of these substances.

Table III. Forced breathing experiments.

Date	Time	Time mins.	Volume c.c.		NH_3 c.c. 0·1 N		Total N Mg		NH_3 N p.c. of total N
			Total	Per hour	Per cent.	Per hour	Per cent.	Per hour	
4/10/19	9 a.m. to 12.43 p.m.*	223	147	40	17·00	6·72	920	364	2·60
	12.43 p.m. to 1.40 p.m.†	57	245	258	0·00	0·00	213	550	0·00
5/10/19	9 a.m. to 12.47 p.m.‡	227	268	71	22·00	15·60	852	603	3·61
	12.47 p.m. to 1.47 p.m.†	60	345	345	1·20	4·14	261	900	0·64

The alkalinity of these urines is not given in this table as it was estimated only by titration without removing CO_2. The increased excretion of bicarbonates is shown in the protocol of the subsequent experiment.

* F.B. commenced 12.30 p.m. Rothera, aceto-acetic acid, nil.

† Rothera, aceto-acetic acid, ? ‡ F.B. commenced 12.48 p.m.

Protocol of subsequent forced breathing experiment.

Date 17/3/20. Subject H. W. D.

12.37 Alveolar CO_2 Insp. 5·10 p.c. Exp. 5·09 p.c. Mean, 5·09 p.c.

1.7 Urine A acid to litmus 189 c.c. (10.10 a.m.—1.7 p.m. *i.e.* 64 c.c. per hour). Rothera negative.

1.10 Commenced forced breathing.

1.30 Urine B alkaline 49 c.c. (1.7—1.30 *i.e.* 128 c.c. per hour) containing equivalent of ·219 grams of $NaHCO_3$*. Alkalinity equal to ·053 normal. Rothera negative.

1.33 Feeling thirsty—skin moist. Drank 200 c.c. water.

1.40 Alveolar CO_2 Insp. 1·59. Exp. 1·70. Mean; 1·65.

2.2 Thirsty, sweating. Urine C 43·5 c.c. alkaline (1.30—2.2 *i.e.* 82 c.c. per hour) containing equivalent of ·184 gram $NaHCO_3$*. Alkalinity equal to ·051 normal. Rothera shows moderate amount of acetone bodies.

2.30 Urine D 103 c.c. alkaline (2.2—2.30 *i.e.* ·221 c.c. per hour) containing equivalent of ·154 gram $NaHCO_3$*. Alkalinity equal to ·018 normal. Traces of aceto-acetic acid. Alveolar CO_2 Insp. 1·55. Exp. 1·81. Mean, 1·68.

2.37 Stopped forced breathing.

2.57 Alveolar CO_2 Insp. 4·87. Exp. 4·96. Mean, 4·92.

3.0 Urine E 149 c.c. acid to litmus (2.30—3 *i.e.* 298 c.c. per hour). Traces of acetone bodies.

3.33 Urine F acid 19 c.c. (3.0—3.33 *i.e.* 35 c.c. per hour). Rothera negative.

4.30 Urine G acid 19 c.c. (3.33—4.30 *i.e.* 20 c.c. per hour). Rothera negative.

4.35 Tea.

6.48 Urine H acid 88 c.c. (4.30—6.48 *i.e.* 38 c.c. per hour). Rothera negative

* *Determined volumetrically from CO_2 given off.*

The excretion of $NaHCO_3$ in these forced breathing experiments appears to us to be inconsistent with the theory of renal secretion briefly described by Cushny (8) in the following terms: "The function of the kidney may thus be shortly defined as the filtration of the non-colloid constituents through the capsule and the absorbtion of Locke's fluid through the tubule cells."

During forced breathing the alveolar CO_2 was reduced to between 1 and 2 p.c., but the total CO_2 capacity, and therefore the total "alkaline reserve," as shown by the CO_2 dissociation curve, after drawing a sample of blood and saturating *in vitro*, was not appreciably diminished. As the arterial CO_2 tension was reduced there must have been less $NaHCO_3$ in the plasma, *i.e.* more Na ions combined with proteins and other "buffer" substances[1]. But there was an increased amount of $NaHCO_3$ in the urine. This negatives the view of Palmer and van Slyke (9) that excretion of $NaHCO_3$ depends only on excess of it in the blood. It also seems to negative the idea of filtration through the glomeruli and the return to the blood through the tubule cells of a fluid of constant composition. The "buffer" proteins, like Congo red, would keep back some of the Na ions so that the hypothetical filtrate would contain less $NaHCO_3$ than normal. If, then, a fluid of constant composition were reabsorbed through the tubules, the urine, contrary to our results and to those of Leathes, would become less alkaline, *i.e.* more acid. That the alkalinity is mainly due to bicarbonate is shown by the fact that these urines, collected during forced breathing, when treated with acid gave off from 80–100 vols. p.c. of CO_2.

The diminution or disappearance of ammonia seems to be a normal response on the part of the liver to "alkalosis" and was shown by Hasselbalch (19) and by Haldane, Kellas, and Kennaway (10) to occur in the alkalosis caused by the hyperpnœa due to oxygen want.

The appearance of acetone bodies is possibly due to the fact that they are shielded by some of the alkali and are excreted before the normal oxidative processes occur. It has previously been shown by Stäubli (11) that in diabetic acidosis the administration of 60 grams per day of $NaHCO_3$ caused the excretion of β-hydroxybutyric acid to rise from 17 to 45·2 grams. In our experiments the amount of aceto-acetic acid was so minute that it could not have had any appreciable effect in neutralising the alkali.

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[1] Joffe and Poulton (4) showed that the plasma at a CO_2 pressure of 10 mm. (1·3 p.c. of an atmosphere) contains only 30 vols. p.c. of CO_2 against 53 at 40 mm. pressure (5·3 p.c. of atmosphere). Thus the bicarbonate content was reduced from ·023 N to ·013 N.

According to the results of Parsons(12) the P_H of H.W.D.'s arterial blood must have been increased on the occasion when his mean alveolar CO_2 reached its lowest recorded value of 1·42 p.c. from a normal 7·41 to 7·79, or by ·38. There would however have been comparatively little alkalosis in the tissues, as Yandell Henderson has shown in numerous papers on acapnia and shock that forced breathing decreases the peripheral circulation. The bad effects noticed by us were relieved by inhalation of oxygen and were presumably due partly to increased stability of oxyhæmoglobin, partly to vaso-constriction.

Effects of ingestion of $NaHCO_3$. On eight occasions H.W.D. (weight 75 kilos) or J.B.S.H. (weight 97 kilos) ate quantities of $NaHCO_3$ varying from 30 to 57½ grams. It was found best to take the bicarbonate shortly after a massive breakfast as otherwise it acted as a strong purgative. Enough water was drunk to wash down the $NaHCO_3$ and to satisfy thirst. The following were the principal results observed:

(1) Increase of the CO_2 capacity of the blood, demonstrating the presence in it of additional $NaHCO_3$.

(2) Increase in the alveolar CO_2.

(3) Moderate diuresis.

(4) Rapid excretion of $NaHCO_3$ in the urine, the concentration rising to a definite maximum in each experiment which rarely exceeded 1/3rd normal or 2·8 p.c. The rate of elimination rose to 7·3 grams per hour.

(5) Great decrease or complete disappearance of the NH_3 in the urine.

(6) Sometimes but not always the appearance of acetone bodies in the urine.

Most of these results are illustrated by Figs. 2 and 3 and by Table V. The weight of bicarbonate excreted is given as $NaHCO_3$ though doubtless some of it was present as $KHCO_3$. The bicarbonate in Fig. 2 was estimated by titration, in Fig. 3 by measuring the CO_2 given off.

The results of experiments on the CO_2-capacity of the blood are given in Table IV and in Fig. 1. The rise in this varies from 5 to 19 volumes p.c. and in one case there was a small but probably significant excess after 24 hours. The increase of 19 volumes in the total CO_2 capacity (*i.e.* the capacity at a high pressure) corresponds to an increase of ·071 gram of bicarbonate per 100 c.c. or 3·2 grams in the whole blood of H.W.D. whose blood volume is about 4½ litres. At the time this blood was taken 41·5 grams remained in his body, and taking the weight of his skeleton as 12 kilos we should expect to find 3·0 grams in his blood

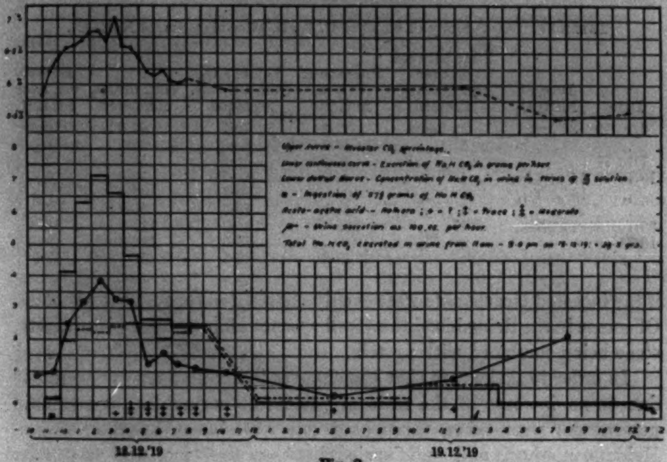

Fig. 2.

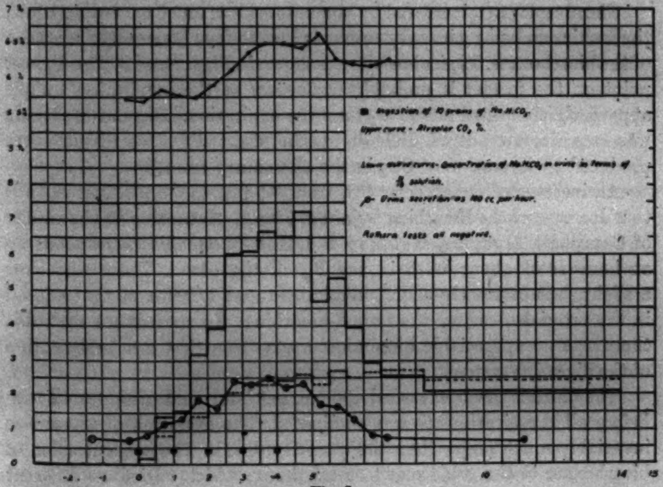

Fig. 3.

Lower continuous curve. CO_2 given off on adding acid per total half hour's urine in 100 c.c. Total $NaHCO_3$ excreted in urine from 0—14 hours = 31·46 grms.

TABLE IV. CO_2-capacity of blood of H. W. D. after ingestion of $NaHCO_3$.

Date	Dose of $NaHCO_3$	Time between 1st dose and drawing of blood	Pressure of CO_2 in mm. Hg	Vol. of CO_2 from 100 vols. of blood	Vols. of CO_2 combined	Excess vol. over normal
6/10/19	30 grms.	2¼ hours	71·3	75·7	70·9	10
„	„	5 „	60·2	66·3	62·2	5
7/10/19	„	24 „	46·3	56·3	53·1	2
10/10/19	15 at 0 hr.	1 „	41·3	60·7	58·0	9
„	15 „ 1 „	4 „	405	130·8	103·6	6·5
„	15 „ 2 „	6 „	41·1	58·5	55·7	7
30/10/19	45 grms.	3¼ „	339	134·0	111·2	18
10/11/19	„	2 „	345	135·5	112·3	19
„	„		43·5	66·4	63·5	13·5

if the ingested bicarbonate were evenly distributed throughout his body. Of course in the living blood the alkali was not all present as bicarbonate, some being combined with protein.

In order to make sure that the changes in alkaline reserve were not due to an increase of the ratio of plasma to corpuscles, hæmoglobin percentages were taken throughout one experiment by the Gowers-Haldane method, and only varied between 94·5 p.c. and 96·5 p.c., although the CO_2-capacity increased by 18 volumes p.c.

The alveolar CO_2 increased by amounts seldom much exceeding 1 p.c., the maximum being generally reached three to four hours after ingestion of the bicarbonate. Typical results are shown in Figs. 2 and 3. The highest point on the former is probably erroneous, as the inspiration sample apparently contained more CO_2 than the expiration. Regular breathing was sometimes rendered difficult by the slight digestive disturbances produced by liberation of CO_2 in the alimentary tract.

An increase of the alveolar CO_2 from 5·5 p.c. to 6·9 p.c. corresponds to a decrease of the breathing by about 15 p.c. According to the results of Campbell, Douglas, Haldane and Hobson (13) this corresponds to an increase of about ·0018 in the P_H of the blood. There was thus a slight, but extremely slight, alkalosis, about 1/200th of that caused in the arterial blood by the forced breathing. In accordance with this there was no appreciable discomfort apart from that due to irritation of the digestive system.

The diuresis never exceeded 400 c.c. per hour, and disappeared long before all the bicarbonate was eliminated. The excretion of water was invariably much larger than the quantity drunk. The concentration of bicarbonate in the urine rose to a maximum which varied on different dates, those of H.W.D. being ·308 normal, ·308 N and ·374 N, those of J.B.S.H. ·358 N and ·269 N. This is in accordance with the results of Ambard and Papin (14), who in a similar experiment obtained a maximum concentration of ·31 N for total sodium salts.

TABLE V.

$NaHCO_3$ ingested	Urine collected	NH_3 c.c. 0·1 N		Total N Mg		NH_3 N p.c. of total N	Rothera
		Per cent	Per hour	Per cent.	Per hour		
11.25 15 grms.	8.15—11.30	21·5	11·2	873	456	3·440	nil
12.25 15 grms.	11.30—12.30*	1·50	2·47	419	691	0·501	nil
1.25 15 grms.	12.30— 1.30*	0·40	0·74	357	660	0·160	nil
	1.30— 2.30*	0·00	0·00	364	575	0·000	?
	2.30— 3.30*	0·80	1·06	419	557	0·270	faint
	3.30— 5.30	0·80	0·66	621	509	0·180	strong
	5.30— 6.30	2·20	1·54	861	603	0·360	trace
	a.m. next day 8.30—12.30	5·10	2·40	966	456	0·739	nil

* Diuresis.

The maximum concentration was gradually attained during the latter part of the diuresis or just after its close, but the concentration does not fall appreciably until many hours after the diuresis has passed. The particular value of the maximum reached did not appear to depend on the magnitude of the dose of bicarbonate or on any other obvious cause. The rate of elimination was greatest during the diuresis, and was roughly proportional to the increase in alveolar CO_2. The greatest quantities eliminated in an hour were 5·80 grams by H. W. D. and 7·32 grams by J. B. S. H. The former represents more than twice the total excess of alkali in his entire blood. In the experiment recorded in Fig. 2, 39·5 out of 57·5 grams were eliminated in the first 10 hours, in that illustrated in Fig. 3 31·46 out of 50 were eliminated in the first 14 hours, yet the concentration had fallen very little. Certainly the excess of alkali in the plasma must have been much less during the early part of the diuresis and yet the concentration was higher. This is in accordance with Ambard and Papin's conclusion—that the kidney requires a certain time to adapt itself to the production of urine of a high concentration, whereas it can produce large quantities of comparatively dilute urine at a moment's notice.

The concentration of ammonia, its rate of elimination per hour, and the ratio of ammonia N to total N all fell rapidly, reaching a minimum and sometimes disappearing completely during the fourth hour after ingestion. Table V gives the results of a typical experiment on H. W. D. It will be seen that the total N excreted per hour remained fairly steady.

Denis and Minot (17) have obtained similar results in cases of nephritis but attempts to duplicate them in normal subjects were unsuccessful. This failure they attribute to excretion of alkali before its neutralising effect on acid radicals has been exerted. They consider speculations regarding "residual ammonia fractions" superfluous, the only function of ammonia being the neutralisation of acid radicals. A similar conclusion has been reached by Janney (18) although he has never succeeded in obtaining ammonia-free urine. The lowest recorded amount of NH_3 nitrogen obtained in one day was ·0086 gram after the ingestion of 60 grams of $NaHCO_3$. Our results confirm the conclusions of these observers and seem to indicate that if sufficient alkali can be introduced into the body all the ammonia will be converted into urea.

Aceto-acetates were generally, but not always, found in the urine. They never appeared in any quantity before the fourth hour after ingestion and their maximum excretion was always after the diuresis and lessened breathing had begun to pass off.

Macleod and Knapp (15) found that lactates appeared in excess in the urine after alkali ingestion. Rough estimations of the lactates in H. W. D.'s urine during one experiment by Ryffel's (16) method seemed to show a slight increase, but exact estimations were not made, as the subject had taken a certain amount of exercise, *e.g.* shaking the blood gas apparatus, during the course of the experiment. In any case the extra lactic acid found would not have neutralised any appreciable proportion of the alkali ingested.

SUMMARY.

1. The complete CO_2 dissociation curve of human blood is given. It does not altogether agree with Parsons' theory at high CO_2 tensions.

2. This curve cannot be altered in man by short periods of forced breathing or breathing moderate excess of CO_2. It is greatly altered by ingestion of alkali.

3. In the acidosis of breathing excess of CO_2 the urine becomes more acid and its NH_3 increases. In the alkalosis of forced breathing or bicarbonate ingestion it becomes alkaline; the NH_3 decreases or disappears; and the rate of bicarbonate excretion may be very rapid.

4. Acetone bodies generally appear in the urine of alkalosis.

5. The concentration of sodium bicarbonate in the urine has a limiting value which is independent of its rate of excretion.

6. The excretion of alkali during forced breathing does not appear

to be compatible with the theory that the kidneys reabsorb a fluid of constant composition from a glomerular filtrate.

7. The alveolar CO_2-percentage is greatly increased by ingestion of alkali.

8. As bicarbonate plays an important part as a buffer in alkaline urine, a special method of titration was employed, and is described, as well as a method for directly determining bicarbonates in urine.

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THE MUSCULAR MECHANISM OF THE DIAPHRAGM.

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In the Arris and Gale Lecture delivered in February 1919 a new view of the action of the diaphragm was put forward by J. C. Briscoe(1). Briefly the proposition was as follows: That the diaphragm should not be considered as a single muscle, nor as constituted of two lateral halves, but should be regarded as consisting in the crura and the two costal portions; the crura acting mesially, and the costal parts arising from the ribs acting laterally. Further, the crura were regarded as the more essential portions, the costal parts being, in a manner, supplementary to the crura. It was claimed that certain exceptional individuals breathed almost exclusively by contraction of the crura, "the crural type," while others, "the parietal type," depended upon contraction of the costal part of the muscle which arises from the ribs. The majority of individuals employ both parts of the muscle in varying degrees during quiet breathing.

Such a division of the muscle into crural and costal portions was supported by a number of considerations. (1) The crural and costal portions are developed from different muscular sheets in the embryo(2). (2) The crura receive their blood supply directly from the aorta; the costal portion from the intercostal arteries and the internal mammary artery. Both sets of vessels communicate with the comes nervi phrenici. (3) The phrenic nerve on reaching the diaphragm divides into two portions. The posterior branch supplies the anatomical crus on that side, and the adjacent muscle arising from the external arcuate ligament (the embryological crus). The anterior branch supplies the remainder or costal portion. (4) A congenital fault in the muscle, when it occurs, is usually found at the point of the junction of these two portions, viz., in the neighbourhood of the last rib. (5) Certain differences were exhibited in X-ray tracings taken from individuals with the two types of respiration and (6) differences were found in the pathological phenomena to which these individuals are liable. (7) Various differences were found in the shapes and joints of the chest, and in the contractions of synergist and antagonistic muscles in these two types.

No direct experimental evidence indicating differences in the contraction of these two portions of the diaphragm had been adduced. The object of this research, was to see whether differences could be detected in the form and mode of contraction of the crural and costal portions, and to find an explanation for such differences if they were discovered.

Certain clinical phenomena had been observed, which if confirmed experimentally, promised to give conclusive support to the theory propounded. (1) Extension of the spine induced the crural type of breathing. (2) Flexion of the spine initiated the parietal form of breathing. (3) The clinical fact that patients dying with ascites were found not to have deflation of the lower lobes (expansion of the latter being attributed to the costal portion of the muscle) led to the conclusion that non-irritative distension of the abdomen promoted parietal breathing.

Methods. Two methods have been followed in recording the movements of the diaphragm. In the first series the animal was pithed and artificial respiration performed. Portions of two or three ribs were removed on one side to allow hooks to be placed on the crus and dome of that side, these hooks being attached by threads to light recording levers. Electrical stimulation was applied to the phrenic nerve, and the movements of the diaphragm on the side exposed recorded. In the second series the animal was kept under ether anæsthesia throughout. A pneumothorax was made on one side of the chest, hooks attached as before to crus and dome, and the movements of the diaphragm with spontaneous respiration recorded. It was sometimes necessary to put in a tracheotomy tube and keep up artificial respiration for a short period if the heart showed signs of flagging. When the heart revived, spontaneous respiration was resumed. Most of the experiments were carried out with the animal lying flat on its back, but the same results were obtained with the animal in a prone position. When lying prone, portions of the 7th and 8th ribs posteriorly were removed. The animals used were chiefly cats and a few rabbits.

Comparison of movements of crural and parietal portions of the diaphragm. The word "dome" is used to denote that part of the diaphragm which arises directly from the ribs or cartilages. Usually the contraction of the crus imparted a larger movement to the lever than that of the dome. The amount of movement, however, could be altered by changing the position of the hooks, so that the contractions, under different circumstances, can only be compared when the hooks are kept in the same relative positions.

When the animal was lying flat on its back with the spine neither flexed nor extended, it was found, in the majority of cases, that the latent period of contraction was shorter in the crus than in the dome. This difference in the latent period was found, both when the contractions resulted from stimulation of the phrenic nerve (Fig. 1), and when they occurred during spontaneous respiration. Records of single spontaneous contractions were obtained by making the drum rotate rapidly during the period of one respiration. These tracings showed that the inspiratory contraction started earlier in the crus than in the dome.

Fig. 1. Contractions of crus and dome, with electrical stimulation of the phrenic nerve.

Effect of flexion and extension of the spine. When the spine is flexed, the contraction of the dome is usually increased, and that of the crus diminished. When the spine is extended by placing a couple of dusters under the animal's back the contraction of the dome is diminished, and that of the crus increased. In all the animals tested, the same type of result was obtained. Although in some cases the contraction of both crus and dome might be increased or diminished by the change of position, the alteration was in the same direction, *i.e.*, the crus was increased relatively to the dome by extension, and the dome increased relatively to the crus by flexion (see Fig. 2). The same results were obtained when the phrenic was stimulated and a single contraction recorded. These tracings showed that the change of position had an effect, not only on the energy, but also on the latent period of contraction. When the animal was lying flat on its back, the latent period of the dome was longer than that of the crus. When the back was extended this difference in the latent period became more marked; when flexed the difference in the latent period disappeared. It would appear then, that extension of the spine not only increases the energy, but shortens the latent period of contraction of the crus, while flexion of the spine increases the contraction, and shortens the latent period of the dome.

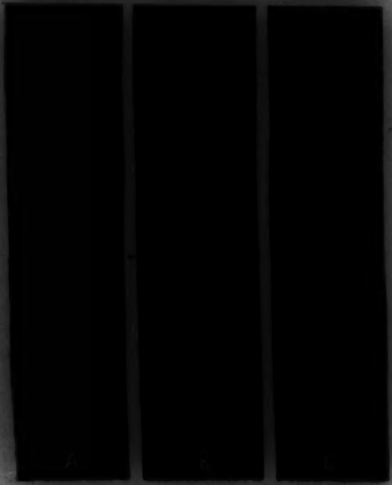

Fig. 2. Cat. Contractions of crus and dome with spontaneous respiration (upstroke inspiration), upper tracing dome, lower tracing crus. *A*. Animal lying flat on back. *B*. Spine extended. *C*. Spine flexed.

The effect of distending the abdomen with saline. In these experiments the animals were lying supine, neither flexed nor extended. The movements of the diaphragm with spontaneous respiration were recorded. As the saline was injected slowly into the abdominal cavity, the movement of the dome lever gradually increased. The injection of saline was continued until the abdomen was tense and the dome pushed up into the thorax. Fig. 3 records the results of one of these experiments. After 260 c.c. of saline had been injected, the amplitude of movement of the lever attached to the costal portion was increased to fourfold the initial contraction. The movement of the crus lever remained practically unaltered.

In a pithed animal, saline was injected into the abdomen, until it was tense. Tracings of a single contraction of the diaphragm, due to stimulation of the phrenic nerve, were taken before and after the injection. The first tracing showed that the latent period of the dome was longer than that of the crus, and that the movement of the dome lever was smaller than that of the crus. The second showed that both crus and dome were now contracting more strongly, with the same strength of stimulus, but that the movement of the dome lever was now greater than that of the crus. The delay in the latent period had also disappeared, the dome now having a slightly shorter latent period than the crus. In short, the effect of distending the abdomen and pushing up the diaphragm into the thorax had been to increase the energy of contraction of the dome, and to diminish its latent period of contraction relatively to that of the crus.

Effect of excitation with varying stimuli. It is obvious when an animal is breathing regularly under anæsthesia, without dyspnœa, that it is responding to a submaximal stimulus. In a pithed animal, the phrenic of one side was stimulated by electrical currents of different strengths, varying from maximal to minimal. The contractions of crus and dome were recorded with the animal lying flat. The experiment was repeated using the same strength of stimuli, with the spine extended and again with the spine flexed. With the spine horizontal, the contractions of both crus and dome were about equal, and the contractions caused by the maximal stimuli had shorter latent periods than those caused by submaximal stimuli. When the spine was extended, the contractions of the dome were considerably diminished, and stimuli which had been submaximal when the animal was flat, now became subminimal and no contraction was recorded with the weakest stimulus employed. When the spine was flexed, the contraction of the dome became much larger and the contraction of the crus less, than in the first experiment. Stimuli which had been submaximal for the crus now became subminimal.

This experiment appears to show that extension of the spine causes a lowered state of excitability in the dome, and that flexion of the spine causes a lowered state of excitability in the crus.

Effect of lateral flexion of the spine. When the spine was flexed laterally, so that the concavity was on the side of the pneumothorax, the contractions of both crus and dome were diminished; when the convexity was on the side of the pneumothorax, the contractions of both parts were increased. On looking at the diaphragm, it was obvious that in the first case, the muscle fibres were relaxed, in the second that they were stretched.

Section of branches of the phrenic. In some cats the phrenic divides into posterior and anterior branches before it enters the muscle. The posterior branch to the crus was cut after a record of spontaneous contractions of the diaphragm had been obtained. The result was that the crus went out of action and the movement of the dome on the same side became less effective (this is illustrated in the latter part of Fig. 3). When the whole nerve was cut, the costal lever still recorded a certain amount of movement. This movement was probably due to the action of the opposite and sound side of the diaphragm pulling over the paralysed side. A similar result was recorded in a pithed animal. After section of the posterior branch, stimulation of the phrenic showed no contraction of the crus, and a diminished movement of the dome. After section of the anterior branches of the phrenic supplying the costal portion, stimulation of the nerve in a pithed animal showed that the contraction of the crus was less effective and that there was no contraction of the dome. In one case, where the contraction of the crus was vigorous, a relaxation of the dome occurred, as shown by the dropping of the attached lever. Probably relaxation of the dome only occurs when the crus contracts fairly sharply and pulls on the unexcited portion of the muscle.

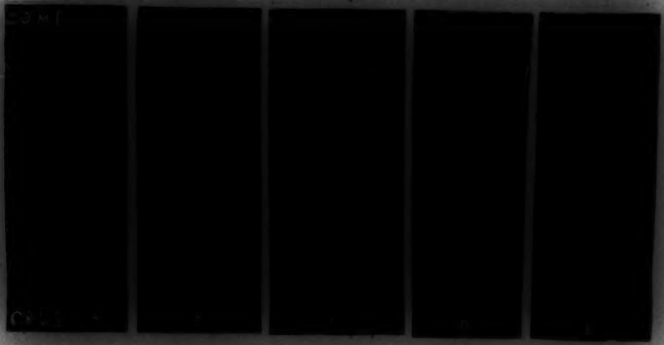

Fig. 3. Cat. Effect of distending abdomen with saline, and of section of posterior branch of phrenic to the crus. *A*. Spontaneous respiration before injection of saline. *B*. 160 c.c. of saline injected into abdominal cavity. *C*. 260 c.c. of saline injected into abdominal cavity. *D*. About 150 c.c. of saline withdrawn from abdominal cavity. *E*. Section of posterior branch of the phrenic nerve, supplying the crus. (The nerve was cut immediately after the record *D* was obtained, without the further withdrawal of fluid.)

The movements of the diaphragm during respiration can be seen best when a posterior pneumothorax is made. The animal lying prone, posterior portions of the 7th and 8th ribs are removed, and through this window both crus and dome can be watched while the movements of spontaneous respiration are being carried out.

The general movement of the whole diaphragm during inspiration is downwards, the greatest descent taking place in the region of the central tendon. With a right-sided pneumothorax the right branch of the trefoil tendon can be seen clearly. When the animal is lying prone, with each inspiration this branch of the tendon descends in its own axis. When the body is flexed, the same part of the tendon comes forward but still descends in its own axis. In consequence, the fibres of the crus are less vertical and more antero-posterior. When the spine is extended, this part of the tendon moves nearer the middle line.

After section of the phrenic nerve on the side of the pneumothorax, the whole of the parts seen became flaccid, except a small portion of the crus round the œsophagus which continued contracting. The paralysed half of the diaphragm was pulled over to the opposite side by the sound half, with each inspiration.

The experiments detailed above all point to the same conclusion—that the differences found in the energy of contraction and in the latent period of contraction are due to the changes in the stretching or tension of the different parts of the diaphragm, brought about by alteration in posture. Extension of the spine produces a relatively increased action of the crus, flexion causes a relative increase in the action of the costal portion. Following the law of skeletal muscle as enunciated by Blix that "the energy of contraction, however measured, is a function of the length of the muscle fibre," it may be assumed that extension of the spine causes a stretching of the fibres of the crus, and that flexion has an opposite effect. That such stretching and relaxation does take place can be demonstrated on the pithed animal by looking through the posterior window. On extending the spine, the fibres of the crus can be seen to be stretched, while when the opposite movement of flexion is made these fibres can be seen to become relaxed. The dome can be observed better through the anterior window. With spinal extension, the dome disappears from view, but with flexion it is pushed up into the thorax, and its fibres are necessarily elongated.

When saline is injected into the abdominal cavity, the energy of contraction of the dome becomes greater as the intra-abdominal pressure is increased, and at the same time the diaphragm is pushed up into the thorax. Here again, the fibres of the costal portion must be stretched.

Probably there is also an increase in the tension of the crus, as in two cases out of four there was a slight increase in the action of the crus; the main effect however was on the dome. The latent period of contraction of the dome becomes shorter as the energy of contraction is increased. Again, with lateral flexion of the spine, the energy of contraction of both crural and costal parts becomes less, when the tension of the muscle fibres is decreased, and greater when the fibres are stretched.

SUMMARY.

The action of the two parts of the diaphragm can be modified by various factors; the modifications are due to changes in the tension of the different parts; and such tension is altered both by the various positions of the body, and by the degree of intra-abdominal tension. The experimental evidence appears to confirm the hypothesis put forward, based on clinical observation, that the diaphragm should not be regarded as a functional whole, but rather that a differentiation should be made between the action of the crural and costal portions of the muscle.

The experimental work was carried out in the Physiological Laboratory in the London School of Medicine for Women, by the kind permission of Dr Cullis. I wish to acknowledge my indebtedness both to Dr Cullis and Dr J. C. Briscoe for help and many suggestions.

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THE AVOIDANCE OF OBJECTS BY BATS IN THEIR FLIGHT.

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(*From the Physiological Laboratory, Cambridge.*)

THREE explanations have been advanced for the way in which bats guide their flight at night avoiding obstacles. First, that they are endowed with very keen sight which enables them to see when everything is dark to man. Second, that their sense of touch is extraordinarily acute, particularly at the wing tips, they can thus almost instantaneously close their wings if an obstacle is encountered during flight.

These two explanations are made very unlikely by the results obtained by Whitaker(1). This author obstructed the eyes of bats with wax and found that while their flight was abnormal, yet they did not run into obstacles about a room. One "flew fairly quickly and kept passing underneath the chairs of which there were over a dozen in the room, it never once, so far as I could see by lying down to watch it, even touched anything with the tip of its wings." "After flying for over twenty minutes it suddenly settled on a chain supporting one of the weights on the gas chandelier, and that it could settle in such a place is in itself, a wonderful proof of the accuracy of this 'second sight'."

A third theory has been advanced, namely, that they possess some sense, the homologue of which is not possessed by man. I have tested the several theories in the following manner. Two rooms of a building had an intervening door. Both rooms were lit with electric light, and had windows provided with well fitting shutters and heavy serge curtains. When the shutters and curtains were drawn, photographic plates could be changed during day light. At night under similar circumstances the darkness was impenetrable. Bats (Pipistrellae?) used to fly into these rooms of a summer evening, sometimes between one and two hundred at a time. They flew as a rule near the ceiling from corner to corner, some faster than others, but all roughly in the same direction. Occasionally one would commence flight the reverse way round the room, when the rest would gradually take up the new direction. During this transition stage, the flight paths became complicated, but although collisions seemed frequently inevitable, they never took place while I was watching. The electric light being on, the shutters were then closed and the curtains drawn. The movements continued as before.

I now took up my position at the electric light switch. With the bats in flight the light was suddenly turned off, the faint swish of the bats' wings continued as before, and no sounds of impacts occurred such as would indicate a collision. On suddenly switching on the light, the bats were seen to be in quite unconcerned flight as if indeed the light had been on all the time.

On several occasions the light was extinguished during one of the transition periods, that is when roughly half the bats were flying one way round the room and half the other, but as before no collisions occurred. On relighting a few minutes later, they were still found to be flying in odd directions, they did not sort out and fly in one uniform direction as they would have done if the light had been shining.

Further experiments were now devised. As stated above, there was between the two rooms an intervening door. If this stood open, the bats would, a few at a time, fly through the opening from one room to the other. If one room were in darkness and the other lit, they flew from one to the other with apparently complete indifference. If the intervening door was gradually closed to six inches fewer passed through and there was a good deal of "fluttering" before the passage was negotiated. If the door was closed to four inches, the bats would as it were come up and look and then fly off without attempting to pass. This experiment was repeated with all light extinguished, and it was found that many bats negotiated the six inch opening though always with much audible fluttering, larger openings than this they swooped through without concern. This fact was found by closing the light switch suddenly and catching one on the point of entering or emerging. Lastly a series of threads were hung across the room, one end being attached to the picture-rail, the other end passing through a hole in a small cardboard box and being then attached to a weight. The interior of the box could be illuminated by an electric flash lamp, a photographer's focussing cloth being placed round the observer's head so as to exclude the light from the room.

With all lights except the flash lamp excluded, and with the bats in flight, no indication was observed (by the bobbing of one of the weights) that any of the bats ran into one of the threads.

These experiments showed that bats in full flight and in what appeared to be absolute darkness can not only steer round a room and avoid one another, but that they can also avoid obstacles such as threads. Further that they can tell whether a door is shut or open wide, or just sufficiently wide open to allow them to pass. Now these facts definitely exclude vision as the sense concerned, and in my opinion they as definitely exclude the sense of touch. For it seems to me impossible that a bat should be able to fly at an object until it touched it and to then avoid hitting it. The sense concerned must be able to perceive objects when they are still a considerable distance away. This conclusion would appear, by the exclusion of vision and touch, to have brought us to the third conclusion, namely, that bats possess some sixth sense not found in the case of man. I am not however prepared to accept that conclusion until another explanation has been tested and proved to be at fault, namely, that bats depend on their hearing for the directional control of their flight at night.

Two properties of sound with which we are familiar as the result of common experience are that sound does not form sharp shadows and that it is not sharply reflected from solid objects. The reason for these effects is that the wave-length of sound is large compared with that of objects with which we are ordinarily associated and therefore diffraction occurs. If, however, the objects are sufficiently large, sound casts shadows and obeys laws of reflection similar to those of light. If sounds of very short wave-length are tested—sounds that are near or above the audible limit for man—then it is found that reflection and shadow formation both occur with ordinary objects. Not only do small objects cast shadows but a flat surface a few centimetres in diameter will reflect these sound waves sharply. Further, quite small objects which would not reflect an appreciable amount of "ordinary" sound reflect short wave-length sound with considerable intensity.

I suggest then that bats during flight emit a short wave-length note and that this sound is reflected from objects in the vicinity. The reflected sound gives the bat information concerning its surroundings. If the path ahead is clear of obstacles, no sound waves are reflected back to the listener. If there are obstacles then these will reflect the sound and the bat will receive an audible warning. Experiments on "sound ranging" apparatus during the war have shown that the sense of direction in man can be made use of for estimating the position in space of objects emitting sound waves. Under ordinary circumstances it is necessary to increase greatly the effective distance between the ears in order to obtain the required accuracy. But if sound waves of sufficiently short wave-length could have been used the same results could have been obtained with the normal distance between the ears. It is highly probable therefore that if a bat made use of short wave-length sound it would be able to estimate the position in space of an object ahead of it with considerable accuracy.

The following facts fit in with this hypothesis:

(1) Bats are known to emit short wave-length sounds near the audible limit of man and above that of some people. (2) Bats have large finely developed and sensitive pinnæ. Many observers have remarked on their acuteness of hearing. (3) Bats are not disturbed by man's speech, but are greatly disturbed if the hands are clapped together or paper is torn (Whitaker). The latter causes them to decrease their speed of flight and to commence "fluttering." The presumption is that while man's speech is below their audible limits, the sounds emitted by the clapped hands or torn paper reach their audible range and disturb the acuteness of audition that is directing their flight.

SUMMARY.

Certain experiments with bats are described in which their flight was observed under conditions which excluded the guidance by vision or touch. The hypothesis is advanced that their flight is diverted by a specialized sense of hearing since the sound waves of short wave-length which they are known to emit are capable of casting shadows and of forming "sound pictures."

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THE ACTION OF THE HCO_3 ION AND OF MORPHINE ON THE RESPIRATORY CENTRE. BY J. B. COLLIP.

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IT has been shown by Scott(1) that there is not the close parallelism between respiration and the C_H of the blood as has been supposed by the theory of Winterstein(2) and Hasselbalch(3). He found that dogs in which the C_H of the blood had been definitely lowered by intravenous injections of Na_2CO_3 responded to increased CO_2 tension in the alveolar air in much the same manner as normal animals. He concluded that undissociated CO_2 acts as a specific respiratory hormone and that the physiological effects of CO_2 on respiration cannot be attributed solely to its acid properties when in solution. Hooker, Wilson and Connett(4) found when the medulla was kept alive by perfusion with defibrinated blood through the vessels of the brain that the respiratory movements of the diaphragm became depressed with a decrease and augmented with an increase in the C_H of the perfusion fluid but that a greater activity of the centre was produced when the perfusion fluid contained CO_2 at a high tension. Macleod and Pearse(5) have interpreted such results as indicating clearly that the carbonate ion (HCO_3) has a stimulating influence on the respiratory centre.

It was noted in the course of an investigation of the effect of induced variations in the alkali reserve of the blood upon the alkali reserve of the spinal fluid, that dogs under constant ether anæsthesia manifested definite respiratory stimulation in certain instances when 5 p.c. $NaHCO_3$ was given by intravenous injection. The amounts of sodium bicarbonate given were such as to preclude the possibility of the C_H of the blood being the effective stimulating agent while the molecular ratio $\frac{H_2CO_3}{NaHCO_3}$ must have been markedly decreased due to an increase in the denominator. Fig. 1 is illustrative of the type of variation in the respiratory activity that was obtained in dogs on different occasions during and after large injections of sodium bicarbonate. The blood-pressure in the left carotid artery was recorded by the mercury manometer. Respirations were recorded by a Marey tambour which was connected to a side tube placed in the tracheal cannula the outer tension of which was kept constant. Injections were made into the left jugular vein by the gravity method. The animals were kept under moderately light ether anæsthesia, the anæsthetic being given by a Woulff bottle. When excessive amounts of bicarbonate were injected it was found that the respiratory movements which were at times first increased, both in amplitude and rate gradually decreased and finally failed. The blood-pressure was maintained at a high level throughout the experiment.

Quite a different picture was presented by dogs which had received morphine by subcutaneous injection prior to etherization. The respiratory movements which were recorded for some time prior to the injection of bicarbonate in order to give an adequate control, were found to decrease as a rule both in rate and amplitude soon after the injection was started. There soon developed however a peculiar periodicity in the respiration which was manifested by short periods of hyperpnœa which appeared at more or less regular intervals. Concomitant with the hyperpnœa there was a marked rise in blood-pressure which was immediately followed by an equally marked fall with a return at once to the normal level. Fig. 2 illustrates the typical periodic breathing and blood-pressure variation which is associated with morphine-ether anæs-

Fig. 1. Dog. Ether anæsthesia. Effect upon respiration and blood-pressure of injection of 150 c.c. of 5 p.c. $NaHCO_3$. (a) Before. (b) At close of injection. Time: 1 second intervals.

thesia and a high level of plasma bicarbonate. The same effect can be obtained with morphine alone (Fig. 3), or with morphine and ether provided the amount of morphine administered is sufficiently great. The bicarbonate in the experiment, of which Fig. 2 is the graphic record, produced an effect which a larger dose of morphine alone would have produced. It would therefore seem to have augmented the action of

Fig. 2. Dog. Morphine 1½ grms. subcutaneous; ether inhalation. Effect upon respiration and blood-pressure immediately following injection of 300 c.c. of 5 p.c. $NaHCO_3$ into jugular vein. *b*, 2 minutes after *a*. *c*, 1½ minutes after *b*. *d*, 1½ minutes after *c*. *e*, 2 minutes after *d*. Time: 1 second intervals.

Fig. 3.

Fig. 4.

Fig. 3. Dog. Periodic breathing following anæsthesia induced by subcutaneous injection of 5 grms. of morphine sulphate.

Fig. 4. Dog. Morphine 2½ grms. subcutaneous followed by ether inhalation. (*a*) Taken at commencement of injection of 650 c.c. of 5 p.c. $NaHCO_3$ into jugular vein. (*b*) Taken 2 minutes after *a*, and during course of injection.

morphine on the medullary centres. Occasionally, however, one may obtain increased respiratory movements in an animal under morphine-ether anæsthesia following the intravenous administration of $NaHCO_3$ (Fig. 4). The injection of small amounts of sodium bicarbonate intraspinally produced intensive hyperpnœa, the onset of which was almost immediate irrespective of whether the animal was under morphine-ether anæsthesia or morphine alone. When spinal puncture was accomplished under local anæsthesia preceded by a small dose of morphine to quiet the animal the same effect was produced. The writer(6) has elsewhere shown that definite stimulation of the respiratory centre follows the injection of very small amounts of sodium bicarbonate solution either into the cisterna magna or the carotid artery.

A rhythmic alteration in the tonus of the cardio-inhibitory centre probably accounts for the peculiar blood-pressure tracing frequently obtained with dogs under morphine-ether anæsthesia with high plasma bicarbonate, as section of the vagi abolished the inhibitory phases.

SUMMARY.

The administration of sodium bicarbonate by intravenous injection may result in increased respiratory activity. The instillation of sodium bicarbonate into the spinal fluid results practically at all times in definite stimulation of the respiratory centre.

The results point to the specific sensitivity of the respiratory centre to the HCO_3 ion. It is possible however that a disturbance in the kation equilibrium in the nerve cells as a result of bicarbonate administration may be the chief basis for the observed stimulatory action of sodium bicarbonate.

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THE ACID-BASE EQUILIBRIUM IN THE CEREBRO-SPINAL FLUID.

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In the present paper we wish to record the results of some experiments we have made on the ionic equilibrium in the cerebro-spinal fluid. The idea of measuring the reaction of this fluid is by no means new. Foa(1), at a time when the electrometric method had been but recently applied to the measurement of the hydrogen-ion concentrations of body fluids, used it for the examination of a sample of cerebro-spinal fluid of the dog, and obtained a value of $0{\cdot}597 \times 10^{-7}$ N for its concentration of hydrogen-ions, *i.e.* a p_{H} of 7·22. The titratable alkalinity of this sample was found to be 0·02 N. On the other hand, Polyani(2) records a determination in which he found a hydrogen-ion concentration of $9{\cdot}084 \times 10^{-11}$ ($p_{\mathrm{H}} = 10{\cdot}0$), a result so far on the alkaline side, as to suggest a complete loss of the combined carbon dioxide of the fluid. As a matter of fact, Mott(3) had just previously called attention to the occurrence in normal cerebro-spinal fluid of about 50 volumes of carbon dioxide per cent., the greater part of which is in firm chemical combination; and from what is now known of the effect of changes of carbon dioxide content on the reaction of other body fluids, for example the blood, it is evident that unless the fluid be collected and examined without loss of carbon dioxide, the observed reaction of the fluid will be much on the alkaline side of that which it possessed in the body. This accounts for the alkaline values, ranging from $p_{\mathrm{H}} = 7{\cdot}9$ to 8·3, obtained by Weston(4), using the dialysis-indicator method and neglecting any possible effect of the carbon dioxide. Hurwitz and Tranter(5) obtained similar values—p_{H} 8·15 to 8·30—as they assumed that all the carbon dioxide was sufficiently firmly combined not to be lost during their experimental manipulations. On the other hand, more recent observers such as Felton, Hussey and Bayne-Jones(6), and Levinson(7), who have taken precautions against the loss of carbon dioxide from the fluid, have found much more acid values of p_{H}, ranging from 7·7 to 7·9 in the case of the first mentioned authors, and from 7·4 to 7·6 in the case of the latter. Lastly, Milroy(8) using the electrometric method, has investigated the changes of reaction of a sample of human cerebro-spinal fluid as its carbon dioxide tension was changed, and has found that in its behaviour it resembles a 0·02 N solution of soidum bicarbonate, made up in 0·18 N sodium chloride.

In the experiments here recorded, we have collected the fluid to be examined in two portions. One sample, the smaller, was collected in such a manner as not to come into contact with air, and on this we estimated the total carbon dioxide content of the fluid as it exists in the body. The second and larger sample of spinal fluid, in which this precaution was not taken, was used for observations at various known tensions of carbon dioxide.

The apparatus we used for collecting the smaller sample is sketched in Fig. 1. It consists of a glass collecting tube (T) about 15 cm. long and capable of holding about 2 c.c. of fluid; at its lower end it is sealed and at the upper is fitted with a short length of pressure tubing (R), provided with a screw-clip (S). This collecting tube is filled by means of a central tube (C), of about 1 mm. bore, which is connected by a light flexible rubber tube to the free end of the lumbar puncture needle. As soon as the latter has been inserted and the cerebro-spinal fluid is seen to drip freely from it, and all the air has been expelled from the needle and the tubing, the end of the small glass tube is placed in the bottom of the collecting tube and as the fluid gradually fills the tube (C) is withdrawn slowly until the collecting tube is completely full up to the clip (S). The central tube is then completely withdrawn and the clip quickly screwed tight. Thus a sample of the fluid can be readily collected which has not come in contact with air, and can then be transported to the laboratory. When required for analysis the fluid is withdrawn into a pipette drawn out into a fine capillary which will reach to the bottom of the tube (T), and from this measured directly into the apparatus for the estimation of its total carbon dioxide content. The main portion

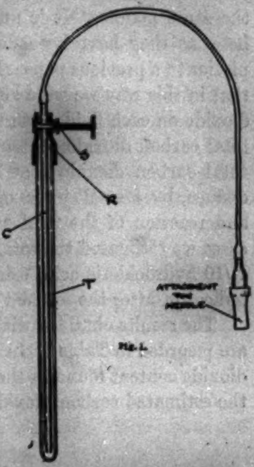

Fig. 1. Apparatus for collecting cerebro-spinal fluid without loss of carbon dioxide.

of the fluid was collected in a test tube without special precautions as to the loss of carbon dioxide. Both samples were preserved on ice until they could be examined.

Of each specimen of spinal fluid separate portions were saturated at body temperature with hydrogen mixtures containing known tensions of carbon dioxide, and then transferred, a part to an electrode vessel filled with the same gas mixture for the electrometric determination of the hydrogen-ion concentration; the remainder to a pipette for measurement into the bottle of the Barcroft differential apparatus, for the determination of the total carbon dioxide content. The alkali used in the differential apparatus was N/40 baryta, and the apparatus was calibrated by the use of a standard solution of sodium carbonate.

The estimations of the total carbon dioxide contents of the specimens of fluid collected from the body without contact with air were made in the same manner. It is unnecessary to give details of these methods here, as they have been described with reference to blood and blood plasma in a previous paper (Parsons(9)). It will be sufficient to mention that in this way we were able to measure, at several tensions of carbon dioxide on each fluid, both the hydrogen-ion concentration and also the total carbon dioxide content. From these values our knowledge of the total carbon dioxide content of the fluid in the body enabled us to deduce, by a small inter- or extra-polation, the carbon dioxide tension and reaction of the fluid as it existed in the living subject. In some cases we estimated the total alkali reserve of the fluid by titration with N/10 hydrochloric acid, using dibromorthocresolsulphonphthalein as an indicator, after the method recommended by McClendon(10).

The results obtained with the specimens of fluid most fully examined are recorded in Table I. In each case the last line gives the total carbon dioxide content found in the fluid in the body and, enclosed in brackets, the estimated carbon dioxide tension and reaction in the body.

TABLE I.

No. of Case Disease	CO_2 tension (mm. Hg.)	Total CO_2 content c.c./100 c.c.	p_H			Alkali reserve
			Observed	Calculated	Difference	
I	31·0	59·3	7·49	7·48	−·01	0·021 N
Syphilis of	41·6	68·6?	7·37	7·40	+·03	
the Cord	(48·0) ? in body	63·0	(7·32)?	—	—	
II	24·1	45·3	$7·54_5$	7·54	−·005	—
Tubercular	41·7	51·4	7·28	$7·28_5$	+·008	
Meningitis	(54·0) in body	55·2	(7·18)	—	—	
III	29·7	44·6	7·41	$7·38_5$	−·025	0·0225 N
Syphilis of	54·3	50·6	7·18	7·16	−·02	
the Cord	(64·0) in body	53·2	(7·12)	—	—	

The first point of interest which arises from these figures is the correspondence between the carbon dioxide combining power and the reaction of the fluids. **Hasselbalch**(11) has shown that in a solution in which the whole of the combined carbon dioxide is in the form of sodium bicarbonate, the p_H of the liquid, the concentration of combined carbon dioxide (Bik.) and that of free (dissolved) carbon dioxide (CO_2) (which is proportional to the carbon dioxide tension) are related by the formula:

$$p_H = p_{K_1} + \log.\frac{(\text{Bik.})}{(CO_2)},$$

where p_{K_1} is a magnitude involving the first dissociation constant of carbonic acid and the degree of ionisation of sodium bicarbonate in the solution. A curve of values of p_{K_1} for various concentrations of combined carbon dioxide is given by **Hasselbalch**: using this, and assuming that the coefficient of physical solubility of carbon dioxide in the cerebro-spinal fluid is the same as that determined for the blood plasma by **Bohr**(12), we have calculated the p_H's of our specimens of cerebro-spinal fluid at the carbon dioxide tensions at which we examined them, and have so obtained the results entered in the column 5 of Table I. It will be seen that the differences between the observed and the calculated values of p_H are less than the experimental error in our measurements, with the possible exception of the second determination made on Specimen I in which our record leads us to suspect that the carbon dioxide estimation has given too high a value. We conclude, therefore, that the fundamental assumptions underlying the application of the **Hasselbalch** formula are justified in the case of the cerebro-spinal fluid, and that the whole of its combined carbon dioxide is in the form of sodium bicarbonate. The same has also been shown to be true of blood, but in blood the factors are complicated by the difference in the carbon dioxide combining powers of the corpuscles and the plasma, so that in this case the application of the **Hasselbalch** formula leads to values of p_H which are consistently lower than those actually observed (**Donegan** and **Parsons**(13)).

The question next arises as to whether the cerebro-spinal fluid behaves towards changes of carbon dioxide tension in the same way as a plain sodium bicarbonate solution, or whether its properties are modified in any way at all similar to that which is so well marked in the blood by the proteins and phosphates it contains. In order to decide this we have, in Fig. 2, compared the reaction changes we have observed in our specimens of the fluid with those occurring in 0·02 and 0·03 N solutions of sodium bicarbonate. These latter curves have been calculated from Hasselbalch's data(11) by one of us on a previous occasion (Parsons(14)).

An examination of this figure shows that the reaction changes occurring in all three specimens of the cerebro-spinal fluid with increasing carbon dioxide tension, are all somewhat more gradual than the corresponding changes in the sodium bicarbonate solutions, but are much more pronounced than those occurring in normal blood, the curve for which is also reproduced in the figure (Parsons(9)).

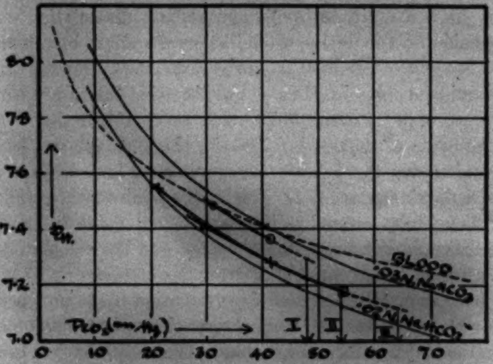

Fig. 2. Comparison between the reaction changes in blood, cerebro-spinal fluid and bicarbonate solutions.

⊙ = experimental determinations on sample I,
+ = " " " II,
□ = " " " III.

I, II and III, represent the conditions of the respective fluids in the body.

RESULTS AND CONCLUSIONS.

The cerebro-spinal fluid is therefore slightly more efficiently buffered than the bicarbonate solutions, but not to an extent at all approaching that exhibited by the blood, in fact the curves for the cerebro-spinal fluids tend to intersect that for the blood, thus confirming Milroy's statement that the fluid is more alkaline than the blood only at low carbon dioxide tensions. There are, then, small quantities of substances in the cerebro-spinal fluid which slightly increase its buffer efficiency above that of a sodium bicarbonate solution of the same alkali reserve. It is probably the smallness of the concentration of such substances in the cerebro-spinal fluid which accounts for the fact that Mott(3) experienced greater difficulty in pumping off its carbon dioxide by heating in a vacuum, than was observed in the cases of lymph and serum, in which the proteins present act as weak acids and expel a larger fraction of the carbon dioxide content. But it is only from whole blood that the carbon dioxide can be driven off completely in this way.

On further reference to Table I, it will be seen that the carbon dioxide tension in the fluid in the body is in each case considerably higher than that in the normal arterial blood, and that similarly the reaction is distinctly less alkaline, the p_H of normal arterial blood being in the neighbourhood of 7·40 (**Donegan and Parsons**(13)). Further, on comparing our results with those of the previous workers on this subject, whom we mentioned in our introduction, it will be seen that we have observed a less alkaline reaction of the cerebro-spinal fluid than they. We regard this as being due to the circumstances that we have taken rigorous precautions to estimate the reaction of the fluid with its normal content of carbon dioxide by adopting the technique we have described, and also that we have carried out estimations at body temperature. It follows from **Hasselbalch's** results(11) that the p_H of a sodium bicarbonate solution containing dissolved carbon dioxide is diminished by 0·10 when it is warmed without loss of gas from 18° C. to 38° C., for under these circumstances the value of the term $\frac{(\text{Bik.})}{(\text{CO}_2)}$ remains unchanged, while the value of p_{K_1} is diminished by this amount, and we have shown that in the cerebro-spinal fluid itself we are dealing with such a carbonic acid-bicarbonate system. In fact our results agree most closely with those of **Milroy**(8), whose technique most nearly resembles our own.

This paper forms a report to the Medical Research Committee. Our measurements were made with apparatus provided out of a grant to one of us (T. R. P.) from the Government Grant Committee of the Royal Society to whom we express our thanks

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THE RELATION OF THE ANIMAL CELL TO ELECTROLYTES. II. The Adsorption of hydrogen ions by living cells.

By J. GRAY, M.A., *Fellow of King's College, Cambridge.*

UNDER normal conditions the egg of the Trout lives in water containing a very low concentration of electrolytes: the egg itself, on the other hand, has been shown to contain a much higher electrolytic content (Gray(3)). The following experiments were performed in order to study the possible uptake of electrolytes when the eggs are exposed to such substances in abnormally high concentrations.

At the present moment positive evidence of such an absorption has only been found in the case of the hydrogen ion and the hydroxyl ion. When normal eggs are exposed to very weak solutions of inorganic acids, it is simple to show that a very considerable amount of hydrogen ion is removed from the solution. If a few eggs are placed in 10 c.c. of distilled water containing sufficient hydrochloric acid to give a bright red colour with methyl orange, the reaction of the water rapidly changes until a distinct alkaline reaction is obtained with the same indicator. In an experiment in which 100 eggs were placed in 40 c.c. of ·005N HCl the reaction to methyl orange changed in half-an-hour from bright red to yellow, indicating a change in the concentration of hydrogen ion to about P_H 4. That these changes are not due to the normal production of such basic substances as ammonia, is shown not only by the subsequent experiments, but also by the fact that the normal evolution of ammonia by such an amount of eggs in half-an-hour is totally inadequate to account for the change in the hydrogen ion concentration of the solutions.

In a very large number of experiments the amount of hydrogen ion removed from solution was estimated volumetrically by means of standard alkali. All the experiments gave clear evidence of a decrease in the hydrogen ion concentration effected by the living cells; the following may here be given as examples (cp. also Table IV).

Data of considerable importance were obtained by estimating the reduction in the acidity of the solutions by a measurement of their

TABLE I.

No. of eggs	c.c. of acid used	Amount of acid present in the solution in terms of standard alkali (N/150 NaOH): Before addition of eggs	Amount of acid present in the solution in terms of standard alkali (N/150 NaOH): One hour after addition of eggs	Reduction in amt. of acid caused by the eggs (estimated volumetrically)
60	40	30·4	16·4	14·0
60	40	84·8	60·0	24·8
95	40	57·3	35·1	22·2
86	40	28·2	16·8	11·4

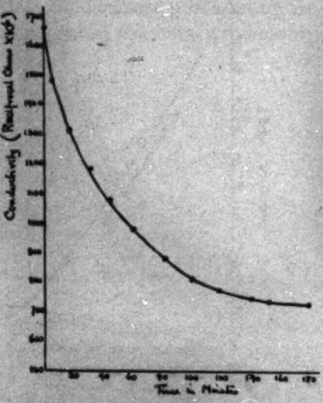

Fig. 1.

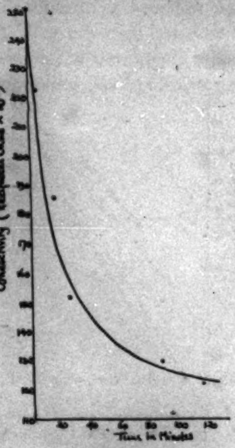

Fig. 2.

Fig. 1. Change in conductivity of 40 c.c. ·003N (approx.) hydrochloric acid solution when in contact with 200 unfertilised eggs.

Fig. 2. Rate of change in conductivity of 0·7 c.c. HCl solution produced by presence of one egg. (NOTE—The conductivity values in this experiment are comparable to those in Figs. 5 and 9–11 but not to those of other figures.)

electrical conductivity. Fig. 1 shows graphically the changes produced in the conductivity of 40 c.c. of ·003N HCl by the presence of 200 unfertilised eggs of the Rainbow Trout. It is quite clear that the eggs produce a marked change in the constitution of the acid solution and that the rate of this change rapidly falls off after the beginning of the experiment. A similar change has been observed by Miss Hind(6) in the case of vegetable tissues exposed to mineral acids. We can assume at the moment that these experiments confirm the conclusion that the hydrogen ion concentration of the external medium is reduced by contact with the living eggs.

By means of a special conductivity cell, which was very kindly lent to me by Prof. V. H. Blackman[1], it was possible to observe the absorption of hydrogen ion by single eggs from very small quantities of solution. It will be seen from Fig. 2 that similar results were obtained to those of the preceding experiments.

Neither the electrical nor the volumetric data alone, however, afford evidence to show whether (1) the acid is actually absorbed by the cells, or (2) neutralisation takes place by an exosmosis of a base from the cells, or (3) there is an interchange of a metallic ion from the cells with the hydrogen ion of the solution. It is true that the nature of the curve in Fig. 1 indicates that the process is not a simple diffusion of basic excretory products, since the reaction changes its velocity in such a marked manner. Important information is obtainable by a correlation of the electrical and volumetric data.

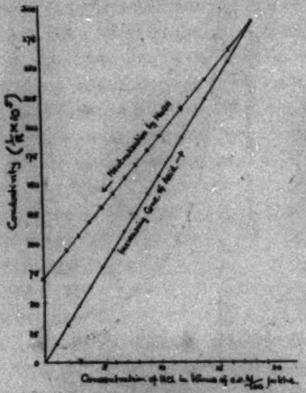

Fig. 3.

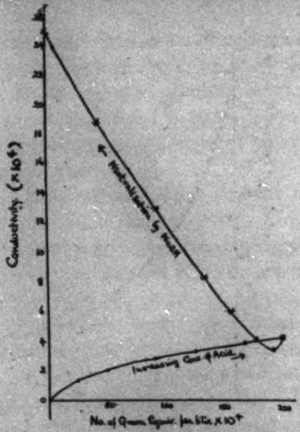

Fig. 4.

Fig. 3. Change in conductivity of hydrochloric acid solutions during neutralisation, and during progressive dilution.

Fig. 4. Change in conductivity of acetic acid solutions during neutralisation, and during progressive dilution.

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[1] Figured by him in *Annals of Botany*, 32, p. 69. 1915.

When an acid is neutralised by a base, the nature of the change in the electrical conductivity differs from that caused by a progressive removal of the complete acid molecules. In the case of a strong acid such as hydrochloric acid, neutralisation by such a base as sodium hydroxide produces a uniform fall in electrical conductivity until neutralisation is complete (Fig. 3); if more base is then added the conductivity rises uniformly with increasing concentration of free hydroxyl ions. Fig. 3 also exhibits the fact that if the acid is removed as complete molecules, the conductivity also falls regularly until it becomes practically zero on the total removal of the acid. It is obvious, however, that the fall in conductivity produced by the removal of a complete molecule of acid is greater than that produced by replacing one hydrogen ion by one sodium ion. In the case of weak acids a totally different series of changes takes place. Fig. 4 shows the effect of neutralisation and of absorption of acetic acid. It will be observed that neutralisation ultimately causes an enormous increase in the conductivity, whereas absorption causes a fall along a definite unbroken curve. The nature of the complex curve produced by neutralisation is readily explained by the ionic hypothesis and need not be considered in detail. A means is, therefore, available for a further analysis of the effect of living cells on acid solutions.

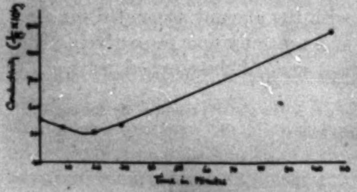


Fig. 5.

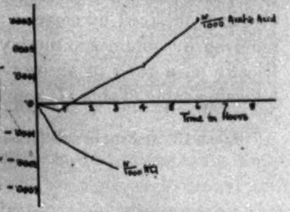


Fig. 6.

Fig. 5. Rate of change in conductivity of 0·7 c.c. N/10 acetic acid solution produced by the presence of one egg.

Fig. 6. Effect of potato tissue on the conductivity of acid solutions (compiled from Hind's data).

Unfortunately prolonged experiments on eggs with acetic acid of satisfactory dilutions for electrical measurements are impossible owing to the toxicity of the solutions. Fig. 5 shows that the changes produced in the conductivity are radically different to those produced in hydrochloric acid solutions. The experiment is, however, somewhat unsatisfactory as the egg showed signs of unhealthiness at the end of the experiment. In this connection the parallelism provided by the work of Miss Hind is most suggestive. Fig. 6 compiled from this author's data, shows most markedly the contrast between the effect of tissues on the conductivity of weak and strong acids. The rise in the conductivity of acetic acid solutions is not therefore due to a simple exosmosis of electrolytes from the cell, but is due to exactly the same causes which produce so profoundly different effects on the conductivity of solutions of strong acids.

There is therefore a very strong suggestion that living cells do not absorb acid molecules as such, but that a neutralisation process of some sort takes place. In the case of trout eggs, this is confirmed by the evidence obtained by correlating the total amount of acid found to be removed by volumetric methods, with the amount accounted for electrically on a basis of absorption and neutralisation respectively. The following table (Table II) summarises some of these experiments.

TABLE II. Amount of acid (in terms of c.c. N/100 NaOH) removed by the eggs in three hours.

		Estimated electrically as		
Strength of original 40 c.c. HCl	Estimated volumetrically	Neutralised by $K^{\cdot}$ or $NH_4^{\cdot}$	Neutralised by $Na^{\cdot}$	Absorbed as HCl
N/500	7·0	8·2	7·5	5·6
N/400	5·7	5·7	5·3	4·0
N/300	10·2	12·7	10·7	9·0
N/200	11·0	12·1	11·0	8·3
N/200	11·0	9·1	8·3	6·2
N/150	14·2	11·1	10·2	7·6
Totals	59·1	58·9	53·0	40·7

It is clear that the amount of acid which is removed from solution as estimated volumetrically is not adequately accounted for by the changes in the electrical conductivity unless we assume that the acid is not removed bodily from the solution, but that some neutralisation process takes place between the egg and the acid. It is clear that if potassium ions or NH_4 ions leave the eggs, and the hydrogen ions take their places, all the experiments are fully explained.

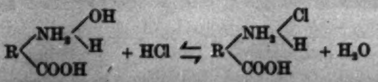
In one series of experiments the change in the concentration of chlorine ions was estimated in addition to that of the hydrogen ion. The chlorine was estimated by the addition of a known excess of standard silver nitrate and the excess of the latter determined by means of standard ammonium thiocyanate solution. With such dilute solutions the results cannot be regarded as of the same accuracy as the determinations of the hydrogen ion, but it is quite clear that the chlorine ion is not removed to anything like the same extent as the hydrogen ion.

TABLE III.

	Original amount of		Final amount in sol.		Amount removed by the eggs	
	H·	Cl′	H·	Cl′	H·	Cl′
	36·8	36·8	18·8	33·3	18·0	3·5
	24·4	24·4	8·0	21·8	16·4	2·6
	18·8	18·8	5·2	16·8	13·6	2·0
	9·4	9·4	2·8	8·95	6·6	0·45
	26·2	26·2	12·8	23·3	13·4	2·9
	18·8	18·8	8·2	16·3	10·6	2·5
Totals	134·4	134·4	55·8	120·45	78·6	13·95

At this point it is interesting to draw attention to the experiments of **Barratt**(1) on the effect of acids and alkalis upon Paramœcium. This observer showed that living Paramœcia absorb hydrogen ions from an acid solution and hydroxyl ions from an alkaline solution, and that these changes were not due to the excretory products of the Protozoa. By means of a calomel electrode it was shown that in the case of hydrochloric acid solutions the chlorine ion is not absorbed at the same time as the hydrogen ion. Finally the amount of hydrogen or hydroxyl ion removed from solution is apparently greater when measured by the potentiometer than when measured by the electrical resistance of the acid solution. The reason for this now appears to be clear.

The results of the experiments with trout eggs is in remarkable agreement with these experiments, but their interpretation is different since **Barratt** concluded that the reaction between the acid and protoplasm was similar to the reaction of an acid with a protein. The usually accepted formula for such a reaction implies that the hydrogen ions and the chlorine ions are bound to the protein radicle in equivalent amounts,



$$R\left\langle\begin{matrix}NH_2\left\langle\begin{matrix}OH\\H\end{matrix}\right.\\COOH\end{matrix}\right. + HCl \rightleftharpoons R\left\langle\begin{matrix}NH_2\left\langle\begin{matrix}Cl\\H\end{matrix}\right.\\COOH\end{matrix}\right. + H_2O$$

which is not the case with the living cell. The present experiments are however paralleled by those of **Hamburger**(5), who observed an interchange of calcium ions and sodium ions between blood corpuscles and serum.

Before discussing this point further a closer examination may be made of the nature of the reaction between the acid and the eggs. As seen from Fig. 1 the eggs practically cease to absorb acid after 2–3 hours.

When the same quantity of eggs are allowed to come into contact with equal volumes of acid of varying concentrations it is found that the total amount of hydrogen ions removed from solution depends upon their concentration in the original solution. In the following table three such experiments are recorded.

TABLE IV.

Exp. 1. 90 eggs in 40 c.c. of solution:

Original amount of H·	Final amount of H·	Amount of H· removed by eggs
7·6	0·5	7·1
9·6	1·4	8·2
12·6	2·4	10·2
19·0	8·0	11·0

Exp. 2. 100 eggs in 40 c.c. of solution:

Original amount of H·	Final amount of H·	Amount of H· removed by eggs
9·6	3·2	6·4
18·8	8·7	10·1
26·2	12·8	13·4
39·2	23·7	15·5

Exp. 3. 100 eggs in 40 c.c. of solution:

Original amount of H·	Final amount of H·	Amount of H· removed by eggs
9·7	2·1	7·6
19·4	5·9	13·5
25·4	9·6	15·8
39·2	13·9	25·3
54·0	17·3	36·7*
78·4	36·8	41·6*

* Eggs showed signs of being unhealthy after the experiment.

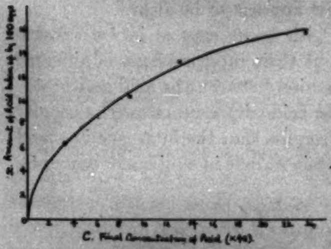


Fig. 7.

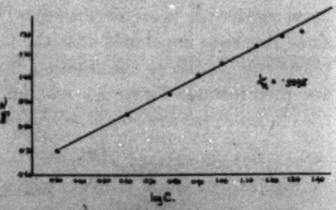


Fig. 8.

Fig. 7. The relation between the amount of acid taken up by 100 eggs and the concentration of the final solution.

Fig. 8. The relation between log x and log C in an experiment with 100 eggs.

Fig. 7 shows graphically the relation between the amount of acid removed by equal quantities of eggs and the amount of free acid left in solution after equilibrium has been established. An analysis of this and similar curves shows that the distribution of the hydrogen ions between the eggs and the solution obeys the usual adsorption equation $\frac{x}{m} = a.C^{\frac{1}{n}}$ or where m is constant $\log x = \frac{1}{n}.\log C$, where x is the amount adsorbed, m is the quantity of absorbent, C is the concentration in the solution after equilibrium has been established, and where a and n are constants. Fig. 8 shows that $\frac{1}{n}$ is practically constant; it varied in different experiments from ·445–·532. Figs. 9 and 10 show that experiments with single eggs gave similar results, although in this case the amount of hydrogen ion adsorbed was measured electrically.

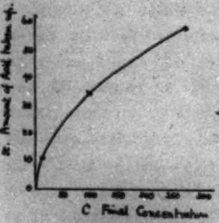


Fig. 9.

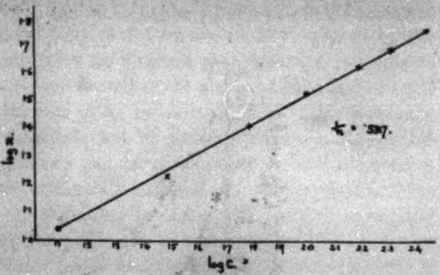


Fig. 10.

Fig. 9. The amount of acid taken up by single eggs from acid solutions of varying strength. [NOTE. The numerical figures are not comparable to those of preceding figures.]

Fig. 10. The relationship between log x and log C in an experiment with single eggs (illustrated in Fig. 9). • Experimental figures. × Figures taken from experimental curve.

In a typical case of adsorption the ion or substance adsorbed distributes itself as described above, and if the equilibrium of the whole system is upset by diluting the final solution with pure solvent, a definite amount of ion or substance goes into solution from the absorbents; in other words the reaction is a reversible one. At the same time the ease with which this reversibility occurs varies greatly in different cases. In the case of living eggs adsorbed hydrogen ions are not given up to distilled water to any appreciable extent. There is, however, evidence that hydrogen ions are given up to solutions containing hydroxyl ions. The experimental evidence is not, perhaps, altogether conclusive since it has been shown that normal eggs absorb hydroxyl ions (see Fig. 11). In the following experiments (see Table V) titration was carried out as quickly as possible. An obvious excess of hydroxyl ions was not allowed to come in contact with the eggs, and since methyl orange was used as an indicator the results obtained can hardly be due to such a cause. The procedure adopted was as follows: eggs washed in distilled water were placed in 40 c.c. of acid of known strength. After one hour 30 c.c. of solution were removed and titrated; the remaining 10 c.c. were titrated in the presence of the eggs. It was noticeable that in the latter case the reaction of the indicator was very slow if the critical concentration of alkali was approached slowly, by adding the latter drop by drop. If sufficient alkali was rapidly added so as just to give an alkaline reaction, the red colour quickly returned, indicating a definite increase in hydrogen ion concentration.

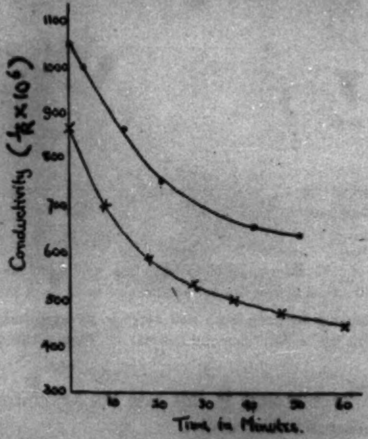


Fig. 11. Rate of change of 0·7 c.c. KOH solution produced by one egg.

TABLE V.

Total initial acid	Total acid in sol. after 1 hr.	Total acid adsorbed	Amt. of equiv. alk. reqd. to neutr. 10 c.c. original sol.	Amt. of alk. reqd. to neutr. 10 c.c. final sol.	Amt. of alk. reqd. to neutr. 10 c.c. final sol. + eggs	Equiv. amt. of acid given up by eggs
38·2	23·2	15·0	9·6	5·8	9·8	4·0
76·4	56·2	20·2	19·1	14·0	26·2	12·2
—	—	—	—	9·5	23·0	13·5

It would therefore seem that the adsorption of hydrogen ions by the eggs is a partially reversible reaction. If we may judge by analogy of the effect of such ions on other living cells, the reversibility of its action can be readily established (Gray(4)).

The fact that living cells react to acids by adsorbing the hydrogen ion and by giving out a corresponding amount of kations, provides an interesting explanation of the results obtained by Loeb(7) with the eggs of Fundulus[1]. When such cells are treated with potassium chloride solutions of toxic concentration, recovery can be effected by exposing the eggs to acids or to certain other salts. No recovery is produced by alkalies, distilled water, or by non-electrolytes. It is clear from Loeb's experiments that the potassium ion is the toxic element, and the present series of experiments shows that such ions are readily replaced by the hydrogen ion; but are not given up unless some similar ion is available in the external medium. Loeb describes the action of salts and of acids upon the cell as the production of a "membrane effect": this term may now be defined as the establishment of an adsorptive equilibrium between the ions of the salt and the constituents of the cell membrane. On this basis the whole of Loeb's experiments are explicable. The behaviour of the eggs of Fundulus to acids and salts is essentially similar to such an adsorption process as is here described for trout eggs or to such a system as filter paper and Congo red (see Bayliss(2)). It should, perhaps, be mentioned that Michaelis and Rona(8) have shown that the hydrogen ion and hydroxyl ion are adsorbed to a much greater extent than other ions; and on the principle that a substance which is powerfully adsorbed will replace one which is less powerfully adsorbed, it is clear that the hydrogen ion and hydroxyl ion will readily substitute those which are normally attached to the cell.

At the same time, the possibility of a definite chemical union between the various ions and the cell surface cannot be excluded. It is possible that the adsorption process is simply the precursor of such chemical union; or that the relation between ions and such complex radicles as the cell constituents is in itself a case of adsorption.

It must not be forgotten that the equilibrium between the living cell and the hydrogen ions in the surrounding medium is probably established by means of the outer membrane of the egg. In comparatively mature fertilised eggs the living embryo can be clearly seen within this outer membrane. If the toxic concentration of acid be determined for such eggs and compared with that for newly hatched alevins, it is found that the latter concentration is very much lower than the former.

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[1] In a previous paper (3) it was pointed out that in many respects the eggs of the trout and of Fundulus are very similar.

The toxicity of acids to alevins is so great that their capacity, whilst alive, for adsorbing hydrogen ions cannot be determined. Consequently it must be regarded as probable that the adsorption of the ion is due to the outer membrane of the egg; a conclusion which is in accord with that of Loeb in his experiments with potassium chloride. The identical results obtained by Barratt with Paramœcium point to the conclusion that the relation of living protoplasm to acids is a reaction of essentially similar nature.

Further theoretical conclusions would be out of place, but it is perhaps permissible to mention that the conception of the cell surface as a loose combination between some complex anion with such kations as potassium provides an explanation of a wide range of observed facts. It may also be suggested that a process of ionic adsorption may be one of the means by which living cells take up electrolytes from dilute solutions, although they themselves contain a much higher concentration of such substances.

SUMMARY.

1. When living trout eggs are exposed to dilute concentrations of hydrochloric acid, the hydrogen ion is taken up, but the concentration of chlorine ions in the external solution remains practically unchanged. To replace the hydrogen ions removed from the solution, a kation (possibly potassium) is given up by the eggs.

2. The uptake of the hydrogen ion by the cell follows the laws of an adsorption process.

3. The equilibrium between the external solution and the cell is apparently established by means of the outer membrane of the cell. This process constitutes Loeb's "membrane effect."

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THE EFFECT OF VARYING THE HYDROGEN ION CONCENTRATION AND OF GUANIDIN SULPHATE ON THE EXCITABILITY OF THE NEURO-MYON OF THE FROG. By MARGARET H. GRANT, B.Sc., M.B., Ch.B.

(*From the Physiological Laboratory of the University of Glasgow.*)

Part I. The Effect of varying the Hydrogen ion Concentration.

Stearns and Janney in 1915(1) maintained that the symptoms of tetania parathyreopriva are due to an alkalosis, while McCann in 1918(2) recorded some experiments which in his opinion showed that an alkalosis causes the symptoms of tetany. As shown by the investigations of Noël Paton, Findlay and others (3) these symptoms are due to the action of guanidin and methyl guanidin in stimulating the efferent neurones of the cord and in increasing the excitability of the peripheral neuro-muscular mechanism, probably of the nerve endings. Experiments carried out in this laboratory by Dr Morris have shown that an increase in the alkaline reserve of the blood, brought about by the intravenous injection of sodium carbonate 1·9 per cent., are accompanied by an increase in the electrical excitability of the muscle nerve of the cat and dog. This, of course, is not necessarily due to a direct action of the alkalosis.

As stated by Bayliss(4), neutrality means the concentration of the two ions H′ and OH′ as they are present in pure water, *i.e.* 1×10^{-7} at 25° C.; and any concentration of H ion less than this means alkalinity and any greater means acidity.

The solutions used in the following experiments were different strengths of the acid and alkaline sodium phosphates (NaH_2PO_4 and Na_2HPO_4) and their C_H was determined by the "indicator" method, using the table compiled by Bayliss(4, p. 189). The fact that the sodium salt was used in both cases eliminates the possibility that any variation in their action might be due to variation of the basic radicle.

The muscle used was the frog's gastrocnemius and the stimulus was obtained through the sciatic nerve by means of the single break induction current. A current of two milliamperes was used in the primary circuit, a milliamperemeter being inserted to determine the constancy of the current. The muscle itself was immersed in a bath containing ·75 p.c. sodium chloride, which could be replaced by the solutions to be tested. The nerve was protected from the fluid in the bath by means of strips of blotting paper laid across the top of the bath and underneath the nerve. The fluid in the bath was well below the level of this protecting layer so that none of the solution could be sucked up by it. The nerve, resting on the electrodes above the layer of blotting paper, could be constantly moistened with the salt solution from a dropper bottle.

Fig. 1.

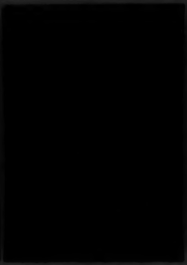

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 1. Contractions in normal saline.
Fig. 2. " $C_H = 10^{-10}$ (1·0 % Na_2HPO_4).
Fig. 3. " $C_H = 10^{-8}$ (0·5 % to 0·125 % Na_2HPO_4).
Fig. 4. " $C_H = 10^{-4}$ (1·0 % NaH_2PO_4).

The tracings were all recorded on a stationary drum, and the first was always taken with the muscle immersed in the salt solution. Immediately after this first trace was recorded the fluid was removed by means of a pipette, and the solution to be tested substituted. Tracings were then taken at regular intervals of five minutes. The lever was, in each case, carefully poised to the same extent, and the muscle was in every way possible put under the same conditions of tension and temperature, etc., in each experiment to obviate any variations that might arise from different degrees of fatigue.

1. *Sodium chloride* 0·75 *p.c.* In the first experiment the muscle was

immersed in the salt solution the whole time, and is the control trace to the rest of the experiments. Several experiments were carried through with each solution but only one of each has been inserted here (Fig. 1).

Alkaline solutions. Fig. 2 shows the results obtained from a series of alkaline solutions beginning with 1 p.c. and going down to ·5 p.c.

Fig. 3 is typical of those obtained from ·5 p.c. to ·125 p.c. solutions, all of which were found afterwards to have a C_H of 10^{-9}.

The tracings obtained from ·0625 p.c., ·03125 p.c. and ·0156 p.c. solutions of Na_2HPO_4 all resembled one taken in normal saline and all were found to have a C_H of 10^{-8}.

Acid solutions. Fig. 4 is an example of the contractions obtained in the acid solutions, in this case 1 p.c. NaH_2PO_4 with a C_H of 10^{-4}.

The tracings obtained from ·5 p.c., ·25 p.c., ·125 p.c. down to ·0156 p.c. solutions of NaH_2PO_4 all resembled those in normal saline and all had a C_H of 10^{-5} to 10^{-6}.

The results may be tabulated as follows:

ALKALINE GROUP.				ACID GROUP.		
1 %.	$C_H = 10^{-10}$	Decrease		$C_H = 10^{-4}$	Decrease.	
·5 %.	$C_H = 10^{-9}$	Increase	10^{-9}	$C_H = 10^{-5}$	Slight decrease	10^{-5}
·25 %.	$C_H = 10^{-9}$	Increase		$C_H = 10^{-5}$	No effect	
·125 %.	$C_H = 10^{-9}$	Increase		$C_H = 10^{-5}$	No effect	
·0625 %.	$C_H = 10^{-8}$	No effect	10^{-8}	$C_H = 10^{-5}$	No effect	
·03125 %.	$C_H = 10^{-8}$	No effect		$C_H = 10^{-6}$	No effect	10^{-6}
·0156 %.	$C_H = 10^{-8}$	No effect		$C_H = 10^{-6}$	No effect	

CONCLUSIONS.

(1) Below 10^{-9} and above 10^{-4}, that is, as the alkalinity or acidity increases respectively, there is a marked decrease in the extent of the contraction of the muscle and a quicker onset of fatigue.

(2) As the acidity diminishes from 10^{-4} towards 10^{-7} the decrease in extent becomes less marked till the contractions resemble those of one in normal saline.

(3) On the alkaline side, however, as the concentration of the H ion increases from 10^{-10} there is marked increase of excitability as seen by increase of extent of contraction with the same stimulus. This lasts throughout the three solutions contained in the group with the $C_H = 10^{-9}$, but when the C_H increases to 10^{-8} the contractions again resemble those in normal saline.

(4) Therefore as the solutions more nearly approach neutrality both from the acid and alkaline side, the contractions come to resemble the normal.

From the foregoing it is thus apparent that the only C_H capable of inducing increased excitability is 10^{-9}. It has already been found that when blood approaches a C_H of 10^{-8} serious symptoms arise, and that when a C_H of 10^{-8} is fully established death results. This at once puts a C_H of 10^{-9} outside physiological limits, and goes to prove that variations of H ion concentrations within physiological limits have no effect on the organism in the way of increasing excitability. It is only when one goes beyond that on the alkaline side to a C_H of 10^{-9} that any increased degree of excitability is obtained, and then the conditions are incompatible with life.

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PART II. THE ACTION OF GUANIDIN SALTS.

In connection with the work done on the effect of variations of hydrogen ion concentration on the excitability of muscle stimulated through the nerve, it seemed of interest to study further the action of guanidin salts which **Noël Paton** and **Findlay** have shown first to increase and later to decrease the electrical excitability of the neuromyon of the mammal. **Meighan**(1) has already recorded some work upon the subject but he confined his observations to its action in producing spontaneous twitchings in the skinned feet of frogs and to the onset of its "curare" action which he found ultimately to appear with concentrations as low as ·02 p.c. **Camis**(2) also tested various strengths of guanidin chloride, but worked on (*a*) the muscle alone, and on (*b*) the denervated muscle. His results, however, on its action on excitability are not conclusive though he states that it may be in the direction of an increase or of a decrease.

In the following set of experiments the sciatic-gastrocnemius nerve muscle preparation of the frog was used and the guanidin salt chosen was the neutral sulphate as in the work of **Putzeys** and **Swaen**(3). The tracings were obtained in exactly the same way as those of the previous set of experiments. A control set of contractions was first obtained by using ·75 p.c. sodium chloride throughout. They resembled those given in Fig. 1 above in every detail. When a 1 p.c. solution of guanidin sulphate was used there was no increase in excitability but rather a fairly rapid decrease followed by the onset of the "curare" action described by **Meighan**(1) (cp. Fig. 5).

Records were then taken with a ·5 p.c. and a ·25 p.c. solution of guanidin sulphate. Both showed marked increased excitability and again towards the end of each experiment the "curare" action made itself apparent (Figs. 6 and 7).

Other two experiments were then carried through using ·0625 p.c. and ·0156 p.c. solutions respectively. The tracing in both cases resembled those taken in sodium chloride solution.

Fig. 5.

Fig. 6.

Fig. 7.

Fig. 5. Contractions in 1·0 % guanidin sulphate.
Fig. 6. " in 0·5 % to 0·25 % guanidin sulphate.
Fig. 7. " in ·125 % guanidin sulphate. This solution also gave a trace showing marked increase of excitability.

CONCLUSIONS.

1. Increased excitability was only obtained with three strengths of the solution, viz., ·5 p.c., ·25 p.c., and ·125 p.c., this increase being followed later by a "curare" like effect.

2. A greater percentage than ·5 caused rapid onset of the "curare" action while a percentage less than ·125 gave results resembling those in ·75 sodium chloride. These results show some relation to those obtained by **Camis**(2) on the contractility of muscle in that the action is depressing for high concentrations but is an exciting one for lower concentrations.

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THE FOUR PHASES OF HEAT-PRODUCTION OF MUSCLE.

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(*From the Physiological Laboratory, Cambridge.*)

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When a muscle is stimulated isometrically it passes gradually into a new elastic condition: as stimulation is continued the elastic condition continues to change up to a certain limit, after which it remains constant; when stimulation ends, the muscle reverts gradually to its original elastic state. Expressed in another way the muscle, on excitation, gradually develops elastic potential energy: as the excitation proceeds this potential energy reaches a constant value; when the exitation ends the potential energy disappears. Finally, in the presence of oxygen there occur certain recovery processes accompanied by an evolution of heat and restoring the muscle to its previous internal condition (1). These four stages, viz. the development of the mechanical response, its maintenance and its disappearance, followed by the oxidative recovery from activity, will be referred to below as the four phases of muscular contraction. The questions arise "how much of the total heat-production is to be associated with each phase and how is it distributed in time?" The experiments described in this paper represent an attempt to answer this question. The investigation arose originally from the simpler question, "what happens to the potential energy of a muscle excited isometrically, when the muscle relaxes?" There are clearly two possible answers: (i) that the potential energy, if not utilised in doing work, is re-absorbed more or less reversibly by the muscle for use in a subsequent contraction; or (ii) that it is degraded into heat by processes analogous (say) to leakage, diffusion or neutralisation. The experiments have decided in favour of the second alternative. With regard to the magnitude of the heat-production derived from the potential energy, it was suggested by previous work(3) that in an isometric twitch the potential energy was a comparatively large fraction of the total energy liberated in the earlier stages of contraction and relaxation, *i.e.* in the initial rapid processes as distinguished from those associated with oxidative recovery; if this were so then the heat liberated at the expense of potential energy during the irreversible processes of relaxation should also be a comparatively large fraction of the total heat-production. This deduction also has been to some extent confirmed. During the *development* of tension in an isometric contraction both heat and potential energy are being produced by the muscle: during the maintenance of the tension (as in a tetanus) heat alone is being liberated: then apparently there comes a short gap during which no appreciable heat is liberated and the tension begins to fall off: next occurs a considerable evolution of heat derived from the potential energy lost in relaxation: and finally the onset of slow oxidative recovery processes leads to a further evolution of heat and presumably to the rebuilding of some chemical or physical system containing free energy available for subsequent contractions. It should be understood that the heat produced during and immediately after relaxation is in no way connected with that associated with these much slower processes of oxidative recovery: the former occurs to exactly the same degree in the absence of oxygen, and is a physical sign of the physico-chemical processes conditioning—or consequent on—relaxation itself, quite apart from whether recovery occurs or not. On the question of what the actual mechanism of relaxation is no direct light is thrown by these experiments: it is clear however that relaxation is accompanied by physico-chemical processes involving a production of heat in exactly the same way as the development and maintenance of contraction are, and this fact may help to direct us in our search for the actual details of the process.

1. Description of apparatus and methods.

A. Galvanometer. In following the complex course of the evolution of heat consequent on stimulation it is desirable to employ instruments recording the rise of temperature with as little lag as possible. A certain amount of delay necessarily arises in the communication of heat from the muscle to the thermopile, but with most galvanometers a much more serious delay results from the slow response of the galvanometer to the E.M.F. generated by the thermopile. It was desirable therefore to employ a galvanometer possessing the required "volt-sensitivity" and having as rapid a response as possible. The thermopiles employed in this work have a limited number of junctions and therefore a comparatively low resistance, and it is unsatisfactory to employ a high resistance instrument such as an Einthoven galvanometer. The instrument employed was the large astatic Paschen galvanometer made by the Cambridge and Paul Instrument Company. This has been found extremely satisfactory (except for a periodic vibration which is sometimes present) and has made the investigation fairly simple. Our cordial thanks are due to the Cambridge and Paul Company for their continual courtesy and help in the various alterations we have made in the instrument.

The instrument is of the moving magnet type, the 26 magnets being arranged astatically in two groups of 13 at the opposite ends of a fine glass rod. The complete magnet system with mirror weighs about 30 milligrams. In order to obtain increased sensitivity the four coils are wound with six sizes of wire, beginning at the centre with the smallest and finishing with the largest. The sensitivity increases as the square of the period, when the control on the magnet system is varied, and for long periods it is possible to read a photographic record of the galvanometer's deflection to 10^{-11} ampere. Such a sensitivity is not required for these experiments but, by reducing it, it is possible to quicken the response until a dead-beat deflection complete in two or three seconds and readable to one part in 500 is obtained when one millionth of a volt is put into the galvanometer circuit. The resistance of the galvanometer is about 12·3 ohms.

It is necessary to render the instrument dead-beat, as otherwise the oscillations set up spoil the records. After some trials a very successful system of air-damping was adopted in which a fine aluminium vane about 3 × 2 mm. was fixed to the lower end of the magnet system. The damping is varied by bringing this vane more or less near to a small brass plate fixed to the face of one of the bottom coils, by tilting the whole galvanometer forwards or backwards by means of one of its levelling screws; the damping can thus, at any sensitivity other than a very high one, be adjusted so that the movements are just, and only just, dead-beat.

It is desirable to shield the galvanometer from external magnetic fields in order to obtain as stable a zero as possible and freedom from disturbances. Two complete shields made of high permeability dynamo magnet steel were designed and ordered, but these have not yet been received. We have been forced therefore to employ one incomplete shield of soft iron (A, Fig. 1). Since however the galvanometer is being used in the experiments described here at (for it) a relatively low sensitivity the lack of a satisfactory shield has not often been serious: the disturbances caused by moving magnetic bodies or changing electric currents have been eliminated by taking sufficient care.

The illumination is carried out by means of a "4 volt" "half-watt" Osram lamp (B, Fig. 1) used with as high a current as it will safely take. This lamp is as good as the Nernst filament lamp, is much easier to procure and use, and uses much less power. The condenser in front of the lamp is covered over entirely with tin except for a narrow vertical slit, and the image of the slit, after reflection from the galvanometer mirror, is focussed on to a horizontal drum (C, Fig. 1) and condensed to a spot by a cylindrical lens (D, Fig. 1). This spot records the movements of the galvanometer photographically on a strip of sensitive bromide

Fig. 1. Photograph of apparatus employed. A, galvanometer in magnetic shield. B, half-watt lamp in tube. C, drum carrying sensitive paper. D, cylindrical lens. E, shutter. F, microvolt. G, switch and resistance-box. H, vacuum flask containing thermopile. K, resistance-box in stimulating (and warming) circuit. L, leads of 25 volt a. c. circuit. M, potentiometer. N, Lucas contact breaker. P, third arm working time-signal. Q, switch to shutter. S, accumulator working shutter.

paper carried on the drum. The light from the lamp is momentarily interrupted every 1 sec. by a shutter (E, Fig. 1) carried on an electromagnet actuated by a Brodie clock. This system has the advantage that the time marks occur *on* the record itself and thus several records can be taken on the same strip of paper without confusion. In order to prevent the magnetic field of this electromagnet from disturbing the insufficiently shielded galvanometer, the electromagnet in all the later experiments was shielded by two soft iron cylinders. The movement of the surface of the drum was about 2½ cm. per sec. when it was required to analyse the heat-production in the initial stages, but about 1 cm. per sec. when a determination of the recovery heat-production was required. The paper used was 8 cm. wide.

The galvanometer, all its leads, the tables and all the instruments upon the tables are carefully insulated from the ground and from one another and the observer stands upon an insulated board. These precautions are necessary in order to avoid electrostatic or other disturbances caused by a leak of current into the galvanometer.

An arrangement in a box (F, Fig. 1) is provided by which an E.M.F. of one microvolt can be thrown into the galvanometer-thermopile circuit in either direction in order to test the volt-sensitivity of the combination, the degree of damping or the rapidity of movement.

A switch and resistance box (G, Fig. 1) was constructed by Messrs W. G. Pye with copper contacts and terminals (to avoid thermo-E.M.F.'s) and manganin resistances: by turning two vulcanite handles any desired connections can be made with the galvanometer and any desired resistance can be put into the combined circuit. The drum is driven by a belt passing to a Palmer motor, placed as far as possible from the galvanometer in order to avoid magnetic disturbance of the latter. The room is darkened and illuminated by a red electric lamp. In this way the whole of the experiment can be conducted in the dark room and no precautions to prevent fogging of the paper need be taken.

B. Thermopile and muscle chamber. The thermopile employed in the majority of the experiments recorded here was the one shown photographically in Fig. 2, A, B, C and D, and diagrammatically in Fig. 3. It was constructed by one of us (W. H.) on an ivoride frame with wires 0·20 mm. in diameter of gold and nickel, soldered with silver solder. Gold and nickel couples provide a thermo-E.M.F. of about 24 microvolts per 1° C., while constantan and iron couples give about 52 microvolts per 1° C. The specific resistances however of gold and nickel are much lower than those of constantan and iron and the lower resistances partly compensate for the lower E.M.F.'s. The reasons for employing gold and nickel in preference to constantan and iron were: (*a*) they are harmless to living tissues while constantan (which is 60 p.c. copper), if the insulation were not perfect, might conceivably be injurious; (*b*) they are not affected by damp which will injure iron; (*c*) they are excellent substances to work and solder being pliable and refractory to the flame; (*d*) they are comparatively good conductors of heat so a thermopile made with them "settles down" quickly after each record has been made; (*e*) there is the possibility of some trouble arising from a galvanic effect produced by the thermopile when slightly damp even when all the junctions are at the same temperature and this is likely to be less in the case of gold and nickel than in the case of constantan and iron. [We have however since constructed a similar thermopile of constantan-iron which has double the sensitivity and appears to be more suitable for estimating the recovery heat-production, but this was not used in the experiments described here.]

Fig. 2. Photographs of thermopile and muscle chamber with cover removed. A and B without muscle; C and D with muscle inserted. Showing leads to electrodes and thermopile, wooden stopper, rubber tubes to chamber, and brass rod to hold the chamber under the water in the vacuum flask.

The wires of the thermopile were wound as shown in Fig. 3. The "hot junctions" are down the centre in a double row of 50, 2·30 cm.

long; the "cold junctions" lie on the ivoride alternately on either side, there being 50 junctions along each side. Its thermo-E.M.F. therefore is about 2·4 millivolts per 1° C., and its resistance is 5·8 ohms. The resistance of the galvanometer being 12·3 ohms the thermopile can provide about 0·000133 amperes per 1° C., so that assuming it to be possible to read the galvanometer to 10^{-11} amperes it would be possible to read a change of temperature to $7\cdot5 \times 10^{-8}$° C. Such a sensitivity is not required for these experiments, but by reducing the sensitivity of the galvanometer its rapidity of movement can be increased and the high sensitivity of the thermopile therefore is a great advantage in that it leads to a more rapid response.

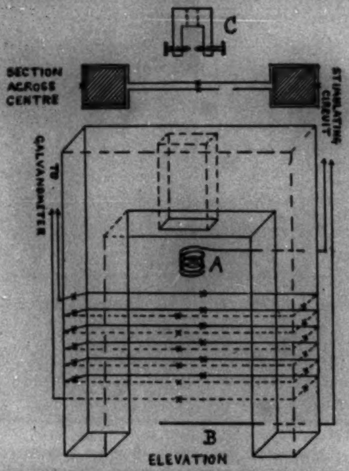

Fig. 3. Diagram of thermopile, perspective and section across centre. A, spiral electrode. B, straight electrode to pass between pelvic ends of muscles. C, clamp to hold bone.

The wires are insulated thickly with shellac to form a solid mass of wires and varnish as shown in Fig. 2, A and B. In order to improve the insulation and to prevent the shellac from becoming "waterlogged" by contact with the wet muscle the thermopile is usually painted before each experiment with a coating of liquid ("medicinal") paraffin. The frame of the thermopile has a hole at its upper end (shown at the top in Figs. 2 and 3) through which to pass the threads from the ends of the muscles. The thermopile carries its own stimulating electrodes (A and B, Fig. 3); the electrode B is a single platinum wire intended to pass between the extreme pelvic ends of a pair of sartorius muscles attached to one another at the bone; the electrode A is a spiral coil of platinum wire through which the two sartorius muscles pass and on which they press firmly so as to make good electrical contact [this is necessary in order to ensure regular results in the control curves obtained by heating the muscles]. The coil is shown round the muscles in Fig. 2, C and D. The leads to the electrodes and to the thermopile are carefully insulated throughout—the platinum electrodes are the only exposed metal in the whole apparatus—and pass to insulated copper wires.

The thermopile is mounted in a vulcanite chamber, milled out from a solid piece of vulcanite, and held in position by a single ivoride bolt and nut. The chamber is provided with a vulcanite cover held on by six screws by means of which it is rendered air-tight. It is also provided with two rubber tubes through which to pass in or out oxygen, nitrogen, Ringer's solution, or any other gas or liquid required. At its lower[1] end it is provided with a movable ivoride rod which is held in a hole through the chamber by a screw, and to which is screwed a clamp holding the muscles. The sartorius muscles of a frog are dissected out and left connected to the bone at the pelvic end. The bone is inserted in the clamp (shown at C, Fig. 3 and in Fig. 2, C and D) and held in position by two small ivoride screws passing into the spherical cavities in it. The clamp is then placed in the chamber below the thermopile and the rod screwed into it. The muscles are then adjusted to lie accurately along the hot junctions one on each side of the thermopile, their ends are passed through the electrode A and the threads to their ends either tied firmly to the frame of the thermopile or passed up through the long metal tube shown in Fig. 2, A and C, to be connected to a lever or other means of recording the mechanical response. The cover is then smeared with vaseline, placed in position and screwed on. The whole muscle chamber containing the thermopile and muscle is then deeply sunk in water at any required temperature, or in a mixture of ice and water, contained in a large double-walled silvered vacuum flask (H, Fig. 1). The copper wires leading to (*a*) the thermopile and (*b*) the electrodes are then connected respectively (*a*) to the galvanometer (through the switch and resistance box G, Fig. 1) and (*b*) to the stimulating apparatus K, Fig. 1, which will be described below. The water inside the vacuum flask is then vigorously and continuously stirred by bubbling air through it from the compressed air plant.

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[1] By "lower" is meant the end actually lower during the experiments, *i.e.* the end shown at the bottom in Figs. 2 and 3.

The development of the combined muscle chamber and thermopile described above makes it possible to avoid, practically completely, all the errors associated with differences of temperature at different points on the thermopile or muscle: the zero is rendered very stable for long periods: it is possible to work at any desired temperature: and the muscles can be bathed in any gas or liquid required during the observation. Thus a variety of experiments is made possible and indeed comparatively simple which previously were liable to serious and sometimes to insuperable error or difficulty; such are for example: (*a*) all experiments in which the muscle is moved or is allowed to move over the junctions; (*b*) experiments on the effects of temperature or at temperatures other than that of the room; (*c*) experiments, such as those on the recovery heat-production, in which a long continued stability of the zero is necessary; (*d*) experiments on drugs, salts or other dissolved bodies in which it is desirable that the muscle should be bathed in a fluid containing the required substances.

In employing this combined chamber and thermopile it has usually been found to be the best practice, especially for the purpose of maintaining the condition of the muscle, to fill the chamber initially with Ringer's solution of approximately the temperature required during the experiment and to blow this out with oxygen only after the chamber has been inserted in the vacuum flask. Nitrogen or air can be substituted for oxygen if required. If it be necessary to keep the muscle in the chamber for some time without using it, it is generally better for the muscle to fill the chamber with Ringer's solution in the interval. Either there is something in the varnish, or the paraffin, or both, which is sometimes slightly deleterious to the muscle, or the favourable effect of Ringer's solution is due to its removal of waste products. After replacing the solution by gas it is necessary to wait for some time (*e.g.* half-an-hour) in order to obtain sufficiently uniform temperature conditions inside. In many experiments of course it is possible to carry out the whole series of observations while the muscle remains in the solution. This is not possible however in all those experiments in which it is necessary to compare the observations on the live muscle with control observations carried out by electrical warming of the same muscle under the same conditions after death: for if the chamber be filled with a conducting solution the warming current will flow mainly through that solution, the heat liberated in the muscle will only be a small portion of the whole heat and the results both in time-relations and absolute quantity will be fallacious. We have carried out experiments on muscles bathed in liquid ("medicinal") paraffin, in order to avoid the presence of gases, and since the paraffin is an insulator the control observations made by electrical warming are valid; we have not pursued the method, however, as it offered no serious advantages for the purpose in hand, and the excess of paraffin appeared to act slightly unfavourably on the condition of the muscle.

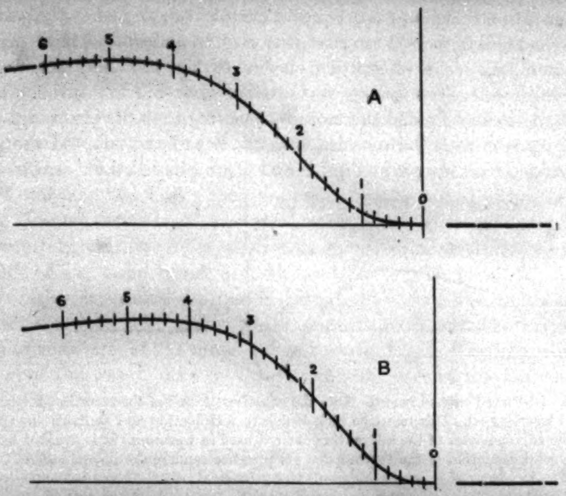

Fig. 4. Normal photographic records of galvanometer response to rise of temperature of muscle on thermopile. A, muscle stimulated while alive for 0·1 sec.; B, same muscle warmed after death for 0·1 sec. *Note:* the records begin at 0, the moment when the stimulus was commenced. The short gaps in the records represent 1 sec. intervals from an arbitrary zero. The lines drawn across the curves and numbered were drawn in afterwards to represent seconds and ½ seconds starting from the moment of stimulation. On examining the curves closely it will be seen that A rises more slowly than B: this however is shown better on the superimposed records of Fig. 7.

The electrical insulation of the thermopile is very important. It is of course desirable, in order to ensure quickness of response, that the insulation of the thermopile should be as thin as possible; if the insulation be too thick the heat may take an appreciable interval to be conducted into the thermopile. Unless however the insulation be good the control observations, carried out as described below by electrical warming of the dead muscle, may be seriously disturbed and rendered quite useless by escape of the current into the galvanometer. A typical disturbed record is shown in Fig. 5 which should be compared with the normal records shown in Fig. 4. The current used is of course an alternating one and theoretically should not affect the galvanometer: unless however the contact between electrodes and muscles is very good a partial rectification of the current appears to occur (see also (3)), and when the galvanometer is reading to 10^{-9} amperes it is easy to understand that rectification and leak of an alternating current of 2×10^{-3} amperes, *i.e.* two million times as strong, may cause serious errors. The insulation of the thermopile can be aided therefore by ensuring that the electrodes make good contact with the muscles, and since we have adopted the spiral upper electrode shown in Figs. 2 and 3 (and have taken care to paint the shellac with paraffin to prevent absorption of water) no serious disturbances as shown in Fig. 5 have been seen. Another reason for ensuring good insulation is that the apparatus is necessarily connected to a source of electricity during the stimulation or control warming of the muscle and this source may have an *electrostatic* potential which if the insulation be leaky, may confer an electrostatic charge on the thermopile and through it on the coils of the galvanometer with consequent serious and sudden disturbances of the records. The insulation of the thermopile has been assisted in this case by electrically "earthing" a certain terminal of the stimulating or warming circuit.

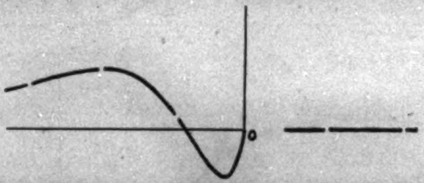

Fig. 5. Disturbed control record. Note the negative "kick" of the record at the moment of warming, O. This negative kick is due to rectification and short-circuit through the galvanometer of the alternating current used in warming. It is avoided by more careful insulation of the thermopile. Its presence renders the record useless.

The material—vulcanite—of which the chamber is made is a good non-conductor of heat so that small temporary variations of the temperature of the water in the vacuum flask in which the chamber is immersed—if such variations occur—do not cause any disturbances in the zero of the galvanometer. The heat-capacity of air is very low, so that the air in the chamber rapidly takes up the temperature of the walls: if however the chamber be filled with Ringer's solution, the heat-capacity of which is high, it will take a long time for an equilibrium to be reached between the inside and the outside unless the initial temperature of the solution is made very close to that of the water in the flask. The large vacuum flasks employed, when filled with some two litres of water, change their internal temperature only very slowly, and this slow change of temperature does not appear to cause any serious disturbances in the thermopile inside the chamber. It is necessary however to ensure that the stirring of the water is adequate, as otherwise small differences of temperature will occur at different depths and such differences have been found to cause serious variations of the zero. The most stable zero of course is obtained by working at an absolutely constant temperature of the water in the flask: such a constancy is obtained either (*a*) by filling the flask with ice and water and working at 0° C., or (*b*) by working at room temperature. It is possible however, without serious trouble, to work at any temperature desired, say from – 5° C. to 30° C.

In order to ensure the better heat-insulation of the chamber its connections with the outside of the vacuum flask, viz. the vulcanite rod and some cm. of the metal tube above it (Fig. 2, A and C), are immersed in the water. The tube passes through a solid wooden stopper which closes the mouth of the flask.

The heat conducting properties of the thermopile are of importance in relation to the type of experiment it is desired to perform with it. It was astonishing to us to find what little hindrance, relatively speaking, a comparatively heavy coating of shellac provides to the conduction of heat from the muscle to the hot junctions of the thermopile. Previous experiments with a Broca galvanometer had shown a considerably greater lag in the response to stimulation or warming, and we had attributed a large part of this lag to the slow conduction of heat into the thermopile: in this we were wrong as the substitution of a galvanometer with much more rapid movements considerably increased the quickness of response. It is clear however from a comparison of the thermo-electric records with those of the deflection obtained when one microvolt is put suddenly into the galvanometer circuit, that a large part of the lag now occurring *is* due to the thermopile. Thus if the insulation can safely be made thinner a still greater quickness of response, with a consequent improvement in the accuracy of the analysis, can be attained.

No less potent than the thickness of the insulation, in determining the character of the response of the thermopile, are the heat-conductivity and size of the wires composing it. If the wires be made very thin, or long, or of relatively badly conducting material (*e.g.* of constantan-iron instead of gold-nickel) the heat will be conducted much more slowly from the hot to the cold junctions. There will be four consequences of this: (*a*) the maximum deflection will be rather larger, as less heat will have been lost from the hot to the cold junctions, and there will be a larger difference of temperature between them at the moment the maximum is attained: (*b*) the maximum deflection will be attained more slowly since the maximum is defined by a balance between the heat conducted in from the muscle and that conducted away to the cold junctions: (*c*) the deflection will decrease less rapidly after the attainment of the maximum as the heat will be lost more slowly: and (*d*) the zero will not be so stable, nor so rapidly attained, as equalisation of temperature will take place more slowly.

In general therefore it may be said that, for convenience and ease of working, a thermopile with a high heat-conductivity, *i.e.* with relatively thick wires of good-conducting material, is the best: but that for recording the heat produced in long continued observations, as *e.g.* in an experiment on the recovery heat-production, a thermopile which loses the heat less rapidly will probably be more effective. It must be borne in mind however that for given metals and for a given number of junctions the lower heat-conductivity will be accompanied by a lower electrical conductivity, with a consequent reduction in the sensitivity.

We have arranged with Mr Wm. Hamilton Wilson, M.I.E.E. of Bank Broadway, Kingston Hill, Surrey, to have these thermopiles made for sale, mounted in suitable chambers. They are being made by him by means of a new process which greatly diminishes the labour of construction and eliminates the need of soldering the junctions.

C. Stimulating and warming arrangements. In order to obtain consistent and regular results, to facilitate the calibration of the system in absolute units of heat and to obtain an alternating current of known period and simple form and at the same time strong enough to ensure that the muscle should be sufficiently warmed by it in a very short interval of time (0·1 sec.), a new type of stimulating and warming system was installed. After the necessary precaution of insulating all the tables, the apparatus and the observer, had been taken this proved accurate and very convenient in use.

The 200-volt single phase alternating current of the Cambridge Electric Supply was connected to a transformer at a distant part of the building and an alternating current at about 25 volts and 90 periods per second was brought to our room on carefully insulated wires. These wires (L, Fig. 1) were connected through an ammeter to the ends of a spiral potentiometer (M, Fig. 1) constructed by Messrs W. G. Pye so that an exactly known E.M.F. (approximately 25 volts) existed between the ends of the spiral. A moving contact enabled one to take off any desired fraction of the whole E.M.F., and this fraction, adjusted as required, was used to provide the stimulating or warming current. The connections are shown diagrammatically in Fig. 6. The stimulating current passed from the potentiometer through a resistance box (in which 1000 ohms was always kept) to a pair of small switches X, to which the leads from the stimulating electrodes in the muscle chamber were connected and returned to the potentiometer through a knock-down key K_2. Another knock-down key K_1 was used as shown in Fig. 6 to short-circuit, when required, the current to the electrodes.

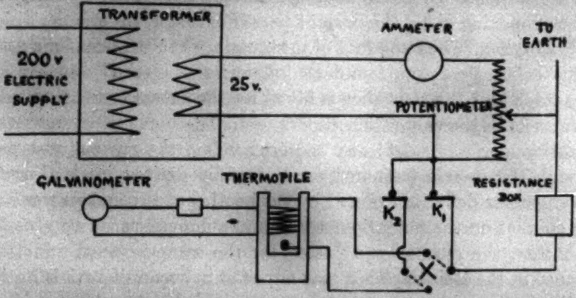

Fig. 6. Stimulating and warming circuit. An accurately measured single-phase alternating current at about 25 volts is brought to the potentiometer, and any desired fraction of the E.M.F. is taken off and used for stimulating or warming the muscle. When the key K_2 is closed and K_1 open the current runs through the muscle. When K_1 is closed as well as K_2 this current is short circuited by K_1. In practice K_1 and K_2 are closed in order. The first arm of the Lucas contact breaker opens K_1 and allows the current to run through the muscle: the second arm opens K_2 and stops the current. During the adjustable interval between the opening of K_1 and K_2 the current passes through the muscle. X is a small commutator-key enabling the current to be passed either way through the muscle, as desired. A resistance is kept in the resistance box to prevent the short circuit key K_1 from absorbing too much current from the potentiometer.

An earth-connection was provided, as shown, to prevent an electrostatic potential in the transformer circuit from producing an electrostatic charge on the coils of the galvanometer: the best position of this earth-connection was found by trial.

The duration of the stimulus (or warming) was determined by the use of Keith Lucas' revolving drum (N, Fig. 1) carrying two movable arms which opened the two electric keys (K_1 and K_2, Fig. 6) at a definite interval of time. The key K_1, until opened by the appropriate arm, short-circuited the stimulating (or warming) current; the resistance of 1000 ohms in the resistance box prevented this short-circuit from absorbing much current from the potentiometer, which had a total resistance of only 31·3 ohms. The second key K_2 when closed allowed the stimulating (or warming) current to pass and when it was opened the current stopped. In the measured interval between the opening of K_1 and the opening of K_2 the current passed through the muscle.

Calibration. The resistance of the muscle, when required for a quantitative calibration, was found as follows: the method has the great advantage that the resistance is found for the actual current traversing the muscle in the calibration experiment. The movable contact on the potentiometer was set to any required value, the current was passed through the muscle (assumed dead) for any desired interval and the galvanometer deflection caused by the heating of the muscle was noted. The contact on the potentiometer was now moved so as to give twice the E.M.F., the current was passed for the same interval and the resistance in the resistance box was adjusted in successive trials until the reading of the galvanometer was the same as before. As there is now the same current as before the resistance of the combined circuit must have been doubled: thus, if the unknown resistance of the muscle be R, and 1000 ohms was originally in the resistance box, the resistance added must be $R + 1000$ and hence R is determined.

The two principal advantages of this method of determining the resistance are: (*a*) no telephone, nor Wheatstone's bridge, nor other extra equipment is necessary; one employs merely instruments already provided for the experiment; and (*b*) the resistance of the muscle does not precisely obey Ohm's law: consequently, for accuracy, it is necessary to find the resistance for the *same* current as was used in warming the muscle. This being done it is possible to calculate accurately the heat liberated in the muscle from the formula $E^2t/4{\cdot}18\,R$ calories.

Time signals. In the observations necessary for the investigation described here it is essential to record precisely on the photographic tracing of the deflection of the galvanometer the moment at which the stimulus (or the warming of the muscle) began. This is done by means of a third key actuated by a third arm (P, Fig. 1) on the revolving drum. The electromagnet working the shutter E which cuts off the light momentarily every 1 sec. is connected to the switch Q by which it can be put into the circuit made up by an accumulator S and the key opened by the arm P. In an experiment the switch is so placed that at first the light is cut off momentarily every 1 sec. by the current sent from the Brodie clock: just before the stimulus (or warming) begins, *i.e.* just before the key K_1 (Fig. 6) is opened, the switch is thrown over by hand so that the shutter is now closed by the current running through the accumulator and through the switch to be actuated by the arm P. A gap in the record therefore occurs. The arm P is adjusted so as to open its key at the same moment as the key K_1 is opened by its arm thereby causing the shutter to withdraw and to allow the record to start again at precisely the same moment as the stimulating (or warming) current begins to pass through the muscle. In this way the record, as shown in Fig. 4, consists of (i) a straight line, with uniform time marks on it, constituting the base line; (ii) a short gap formed by the shutter being switched over into the auxiliary circuit; and (iii) the record of the rise of temperature, starting automatically and accurately at the moment when stimulation (or warming) begins.

As soon as the key is broken by the arm P the shutter is switched back into the circuit of the Brodie clock, and the time signals begin again. With practice the interval during which the shutter is switched into the auxiliary circuit becomes very short, of the order, say, of $\frac{3}{4}$ sec. so that only one of the 1 sec. marks is lost.

The observer working the stimulating (and warming) arrangements is situated some 4 metres from the galvanometer so that his movements cause no magnetic disturbance to the latter. The motor driving the drum is started some time before a record is taken, so that the magnetic field produced by turning on the current in it has no effect on the record. The drum is started about 4 seconds before the stimulus (or warming) begins by pulling a string and thereby releasing a catch. When the long-continued heat-production of recovery is not required, four records are usually made on the same sheet of bromide paper, by arranging for the drum to start from each of four standard positions of the catch.

In order to ensure that the record is made properly on the bromide paper, that the light is suitably adjusted and the spot of light at an appropriate position at the start, it is usual for a second observer to stand near the drum and to watch the excursion of the spot of light. Similarly if a simultaneous record of the mechanical response is being made another observer may work the recording arrangement near the muscle chamber. The whole of the experiment can, however, if required, be managed and worked by one observer.

In dealing with the very sensitive galvanometer used in these experiments and in employing a relatively strong alternating current in the room, it is necessary to take the utmost precautions to prevent leakage of the current into the instruments. For this purpose the instruments were very carefully insulated on paraffin or vulcanite blocks, the observer was insulated from the floor, and he never touched any part of the circuit directly with his fingers during an experiment.

A vibration of the magnet system of the galvanometer of fairly regular period (0·8 sec.) is sometimes, for weeks on end, of sufficient amplitude to destroy the accuracy of photographic records; we have not been able to elucidate the mystery of this vibration, and have been able to work only in its absence.

In order to obtain axes of reference on the photographic record, the drum is revolved against a fixed pencil to give a line round it, and the spot of light is made to record a line across the paper by moving the control magnet.

D. The analysis of the records. If the photographic records of the deflection produced in the galvanometer by the rise of temperature of the excited muscle be examined it will be found (see Fig. 4) that they start off horizontally, bend round gradually, reach a maximum in a few seconds and then return more or less slowly to the original base line. During the later stages the movement is so slow that the displacement from the base line probably represents, fairly accurately, the difference between the temperature of the muscle at the moment and its original value. In the earlier stages however the deflection at any moment does not represent at all exactly the rise of temperature of the muscle at that moment. If the muscle be warmed nearly instantaneously (*e.g.* in 0·1 sec. as shown in Fig. 4, B) the heat has first to be conducted through the insulation of the thermopile, the E.M.F. generated has to produce a current in the galvanometer and the magnet system, affected by its inertia, its control and its damping, has to respond. At the same time the heat is being lost by conduction, convection and radiation, the cold junctions of the thermopile are being warmed by conduction from the hot junctions and the whole system is gradually settling down to its original temperature.

It might be, and it was at first, supposed that the complete determination of the rate of heat-production from the photographic record would involve so many complex factors and such an amount of mathematical analysis as to be practically impossible. On the other hand, without any analysis at all, the differences between the records produced by a live muscle stimulated at 0° C. for 0·1 sec. and those produced by the same muscle after death and warmed for 0·1 sec., made it clear in a general way that a considerable amount of heat was liberated by the live muscle some time after the stimulus was applied (see Fig. 7). The accuracy of the records left no kind of doubt on this point, but for a more exact statement of the phenomena some form of analysis was required. Fortunately one special property of these curves was found to make the analysis of them possible, and it may be said in general that from any record with the aid of a control curve the actual course of the heat-production may be calculated up to any stage desired, and with a fair degree of certainty.

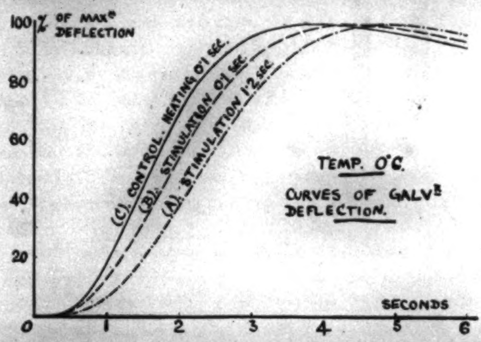

Fig. 7. Superimposed curves of galvanometer deflection, plotted on the same scale of max. = 100 p.c., taken from the same muscles under the various conditions noted. It is seen that the heat is given out appreciably more slowly by the live muscle stimulated for 0·1 sec. than in the dead muscle warmed for 0·1 sec.; while the heat is given out even more slowly in the live muscle stimulated for 1·2 secs. For an analysis of these records see Fig. 9.

The special property of these curves upon which the method of analysis depends is similar to that possessed by curves representing sound waves, tides or other periodic vibrations: viz. that the complex curve can be constructed out of a number of simple curves of the same type and differing only in amplitude, frequency and phase. In the case of our galvanometer records, if we regard the "fundamental curve" for any given muscle, thermopile and adjustment of the galvanometer as being that given by an instantaneous heating of the muscle (actually by a heating lasting for 0·1 sec.) then the special property of these records is that, however complicated, they can be built up additively of a number of fundamental curves differing only in amplitude and phase. The property is made clearer by reference to Fig. 8 where a record is shown built up of a number of fundamental or "control" curves corresponding in size and time to the small rectangles shown above them in black, which represent the quantities of heat produced in successive equal intervals of time.

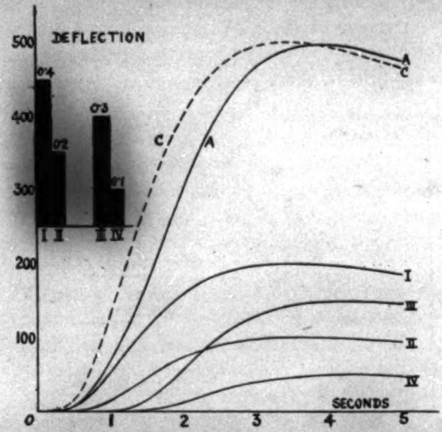

Fig. 8. Curves to illustrate the method of analysis. C is a control curve given by electrical heating of the dead muscle for 0·1 sec. with 1 "unit" of heat. I is a similar control curve (obtained from C by diminishing the ordinates in the ratio 0·4 : 1) starting at time 0 and corresponding to 0·4 unit of heat (rectangle I); II a similar control curve starting at time 0·2 sec. and corresponding to 0·2 unit of heat (rectangle II); III a similar control curve starting at time 0·8 sec. and corresponding to 0·3 unit of heat (rectangle III); IV, a similar control curve starting at time 1·0 sec. and corresponding to 0·1 unit of heat (rectangle IV). A is then obtained by adding the ordinates of I, II, III and IV, and corresponds to 1 unit of heat distributed in time like the rectangles I, II, III and IV.

The task of analysing a record therefore is similar to that of resolving a tide or a sound wave into its several sine-curves. The method, graphically considered, consists essentially of the following process. The first small portion of the record has to be fitted by a control curve, the "fit" being made as good as possible by adjusting the size of the control curve. The difference between the ordinates of the two curves, if plotted, would give a second curve, starting rather later, to which another control curve has to be fitted, the fit again being made as good as possible over the next small portion of the curve by adjusting the size and displacing the position in time of the control curve. The process is repeated, the fit being carried gradually along the whole length of the record by selecting control curves of appropriate size and position in time. The magnitudes and positions of these control curves, into which the record has been split up, are then plotted as rectangles standing on the appropriate time base as shown in Figs. 9–11. Actually in practice the process is carried out numerically and not graphically.

Several control curves (5 or 6 say) produced by heating the dead muscle for 0·1 sec. are made and from the mean of them is constructed a "control table" which is employed as described below in the analysis of the curves given by the live muscle. The method of numerical analysis is best explained by considering an example of the converse process, viz. constructing the curve of galvanometer deflection when known amounts of heat are evolved at known times. In Fig. 8 the curve C is the "control" curve in a certain experiment, this being the curve of galvanometer deflection, drawn to a scale with a maximum represented by 500, when a certain known quantity of heat, called for convenience a unit, is given to the dead muscle by an alternating current passing for a short time (0·1 sec.). Suppose that it is required to find the curve of galvanometer deflection when heat is given to the muscle in the way indicated by the rectangles in Fig. 8, viz. I, at time 0, 0·4 unit; II, at time 0·2 sec., 0·2 unit; III, at time 0·8 sec., 0·3 unit; and IV, at time 1·0 sec., 0·1 unit. The procedure is as follows: the control curve is reduced in height according to the heat given in each of the four cases and the four curves (numbered I, II, III and IV in Fig. 8) are drawn so that each starts from the base line at the point at which the corresponding amount of heat was given. The ordinates of the four curves at each time (or at sufficient times to define a good curve) are then added and the result is the curve A; this curve, as it rises, lies considerably to the right of the curve C because in the latter case all the heat is given at the beginning while in the former case some of it is delayed so that its effect is not felt until later on. It should be noticed that the curve A does not rise to quite the same maximum as the curve C although the quantity of heat given is the same in each case; this is due to the curves I to IV having their maxima at different times. If the heat given in A had been distributed over a longer time than in the case considered this curve would fall further below C, but it will be seen that the curves will differ in maximum height by very little in the two cases (1) when the heat is given out rapidly; and (2) when the control curve is very flat after the maximum (this being so when the thermopile conducts heat very slowly between the hot and the cold junctions). This is important as it means that, in these cases, the maximum height alone of the curve A is sufficient to determine the total heat-production.

In the consideration of the case which occurs in practice, viz. that in which the curves C and A are observed, and it is required to determine the evolution of heat which was necessary in order to produce the curve A, the procedure is not so simple. The curve A has to be broken up into a set of curves all of the same type as C and it is necessary to determine both their sizes and the positions of their starting points so that when their ordinates at each time are added the result will be the curve A. It is best to conduct the analysis numerically.

TABLE I.

Times (secs.)...	0	0·2	0·4	0·6	0·8	1·0	1·2	1·4	1·6	1·8	2·0	2·2	2·4	2·6	2·8	3·0
C	0	1	6	29	73	131	191	254	310	358	399	432	455	473	486	494
0·5 C	0	1	3	15	37	66	96	127	155	179	200	216	228	237	243	247
0·4 C	0	0	2	12	29	52	76	101	124	143	159	173	182	189	194	198
0·3 C	0	0	2	9	22	39	57	76	93	107	120	130	136	142	146	148
0·2 C	0	0	1	6	15	26	38	51	62	72	80	86	91	95	97	99
0·1 C	0	0	1	3	7	13	19	25	31	36	40	43	45	47	49	49
A	0	0	2	13	35	67	104	149	200	251	301	348	386	418	445	465
at 0 sec., 0·4 C	0	0	2	12	29	52	76	101	124	143	159	173	182	189	194	198
subtract	0	0	0	1	6	15	28	48	76	108	142	175	204	229	251	267
at 0·2 sec., 0·2 C		0	0	1	6	15	26	38	51	62	72	80	86	91	95	97
subtract		0	0	0	0	0	2	10	25	46	70	95	118	138	156	170
at 0·8 sec., 0·3 C					0	0	2	9	22	39	57	76	93	107	120	130
subtract					0	0	0	1	3	7	13	19	25	31	36	40
at 1·0 sec., 0·1 C						0	0	1	3	7	13	19	25	31	36	40
subtract						0	0	0	0	0	0	0	0	0	0	0

The first line shows the control curve C by its ordinates at every one-fifth second up to three seconds, this being sufficient to determine the heat-production during the first second or so. The next five lines show the same curve but the size reduced in a given ratio, as shown in the first column; the seventh line shows the curve A which it is required to analyse. The analysis is carried out by subtracting from the numbers corresponding to the curve A the numbers in any of the preceding lines, shifting these latter sets along to the right any number of places as may be required and continuing this process till the final result is as small as possible.

At the beginning of the analysis it may seem doubtful whether the numbers of the A curve, near the start, are more like those of 0·5 C or those of 0·4 C, but proceeding on the suppositions that (1) there is only a positive evolution of heat and (2) that the final result, as regards heat-production, should be as smooth as possible, the latter should be taken; in such cases it is best as a general rule to subtract the less of two alternative sets; if this really happens to be less than should have been subtracted the discrepancy can be rectified by subtracting one of the smaller sets of numbers, starting at the same time, always avoiding negative results as far as possible.

After the numbers for the curve 0·4 C, starting at time 0, have been subtracted from those for the curve A, the resulting numbers show at once that it is much better not to subtract any more of the rows of numbers starting at time 0, but the first few numbers correspond very closely to those for the curve 0·2 C starting at time 0·2 sec.; in this case the first three numbers are completely wiped out and the remainder shows that there is no further evolution of heat until the time 0·8 sec.

In practice, where there are usually many curves to be analysed by the same control curve, it is worth the time to make a much more extensive "control table" than that shown above in the five rows of numbers from 0·5 C to 0·1 C so that there is a wider choice and a better chance of getting a good fit at each stage as the work proceeds. It is also convenient to have this control table on a separate sheet which can be moved laterally relative to the numbers for curve A: in that case only the successive sets of remainders are written down.

The interval between successive times in the analysis of the initial heat-production or of the heat evolved during relaxation has usually been taken as 0·2 sec. or ¼ sec.; if the accuracy of the observations could be improved the interval might be reduced to 0·1 sec. with advantage. For the recovery heat, which lasts several minutes, a suitable interval for the analysis is about 5 seconds.

Mathematically considered, the special property of these curves, which renders the analysis possible, depends upon the fact that all the processes, mechanical, physical and electrical, resulting from the warming of the muscle are governed by linear differential equations with constant coefficients and may therefore be dealt with, so to speak, additively. The extra deflection produced at any time by any quantity of heat liberated at any moment subsequent to stimulation is simply added on quantitatively to the deflection produced at the same time by any other quantity of heat liberated at any other moment. The mathematical proof of this need not be given here, but an experimental proof of it for special cases is easily given by liberating heat electrically in a muscle at any desired rate for any given period and showing that the record so obtained can be built up from the appropriate control curves. An exact agreement between the calculated and the observed curves is then found, showing that the method of analysis is valid and that the special property of the curves exists as stated.

There are of course limits to the accuracy and discrimination to be attributed to the analysis. In the ordinary way it is advisable to assume that the rate of heat-production follows as smooth a curve as possible and when the analysis yields a rather irregular curve to put a smooth curve through it and repeat the analysis. If it be found that the smoothed curve produces at least as good a fit as the original one the modifications necessary to produce the smooth curve may be accepted. Experience only will tell the investigator how far the details of the analysis are reliable in any given case: we have endeavoured however to exclude all experiments in which the results of the analysis did not appear reasonably certain.

2. EXPERIMENTAL RESULTS.

The present part of this paper deals only with the heat produced during the first few seconds after excitation and is confined to the case of tetanic stimulation, for periods of 0·1 to 6·0 seconds, of the sartorius muscles of the frog (Rana temp.). It is clearly desirable to repeat the investigation on other and more slowly moving muscles and we hope to do this later. The experiments have been carried out at various temperatures, in oxygen and in nitrogen, and under various conditions of fatigue.

The first important point brought out by these experiments is that, provided that advanced fatigue be avoided, no difference can be discerned in the initial stages of the heat-production between muscles kept in oxygen and muscles left for a long time in nitrogen. It is known, and in experiments performed recently we have amply confirmed, that in the later stages there is a considerable difference in the heat-production according as the muscles are kept in oxygen or nitrogen; this difference is attributed to slow oxidative recovery processes possible (at least to their full extent) only in the presence of oxygen by which the muscle is restored in some manner to its previous condition.

Oxygen however has no effect whatever on the *initial* heat-production. It is not necessary to give all the evidence in detail as it is negative in kind and we will include below only an account of one experiment devoted specifically to testing this point. The direct comparison of the curves made by the muscle (*a*) in oxygen and (*b*) in nitrogen is an extremely sensitive one, as no analysis of the records is required in order to allow us to assert that the two curves are the same; thus any possible error in the analysis is eliminated. Great care was taken in the nitrogen experiments to eliminate the oxygen completely by sweeping it out as far as possible with boiled Ringer's solution in which the muscles were allowed to rest for some time, then leaving the muscles for some time in nitrogen before making an observation, and lastly giving them (in most cases) a few preliminary stimuli in order to assist them to use up any traces of dissolved oxygen. A mild degree of exercise not leading to appreciable fatigue does not appear to alter the type of the thermal response: advanced fatigue however does change it by diminishing the relative amount of heat liberated in the processes of relaxation. Muscles kept in nitrogen are readily fatigued and it is necessary therefore to avoid over-stimulating them.

Initial heat-production in oxygen and in nitrogen. Protocol.

Pair of sartorius muscles of Frog. Thermopile and muscle-chamber kept at 0° C. in Dewar flask. Stimuli of short duration only were given, to avoid, as far as possible, the effects of fatigue.

The chamber was first filled with oxygen and stimuli of 0·1, 0·2 and 0·4 sec. given; the corresponding galvanometer deflections for various times read off directly from the photographic records and reduced to the scale of maximum deflection = 500 are given in the following table and indicated by the symbol O_2. Each line gives the mean of four sets of readings. The chamber was next filled with Ringer's solution from which the oxygen had been expelled by boiling; nitrogen was bubbled through for ½ hour, and then the Ringer's solution was blown out by nitrogen and the muscles left in nitrogen for 1½ hours.

Stimuli of 0·1, 0·2 and 0·4 sec. were then given and the photographic records of the galvanometer deflection corresponding to the heat-production obtained. To make it more certain that the deflections were not affected by traces of oxygen remaining in the muscles the same stimuli were then repeated (the muscles remaining in nitrogen) and the results were found to be indistinguishable from those previously taken in nitrogen. The galvanometer deflections for various times are given in the following table and indicated by the symbol N_2.

To find the effect, if any, of moderate fatigue the muscles were now given 30 stimuli of duration 1 sec. with intervals of 1 sec. The deflections for a stimulus of 0·4 sec. were then recorded photographically and the result, indicated by the symbol N_2', is shown in the table (mean of two sets of readings). The same process was repeated and the result, indicated by N_2'', is shown in the table.

Finally the muscles were killed with chloroform vapour and the "control" deflections recorded, these being due to giving the muscles a known quantity of heat liberated in 0·1 sec.; these deflections for various times are shown in the first line of the table (mean of four sets of readings).

The last column of the table shows the total heat-production in the first few seconds (and thus does not include more than a very small portion of the heat evolved during recovery); it will be seen that when the muscle is not appreciably fatigued this total heat, for a given time of stimulus, is much the same whether the muscle is in oxygen or in nitrogen; the last two entries in this column show the effect of fatigue by the very considerable reduction in the total heat-production.

TABLE II.

		Reduced galvanometer deflections at various times (in seconds)														Total heat production in millicalories
		0	0·4	0·8	1·2	1·6	2·0	2·4	2·8	3·2	3·6	4·0	4·4	4·8	5·2	
Control. Time of heating 0·1 sec.		0	3	40	112	202	290	363	416	455	480	493	499	499	495	
Stimulus 0·1 sec.	O_2	0	6	34	76	135	209	284	353	408	447	475	491	499	500	0·96
	N_2	0	9	34	76	139	220	294	362	413	454	476	492	499	500	0·79
Stimulus 0·2 sec.	O_2	0	5	30	72	132	206	281	352	406	446	474	491	497	500	1·09
	N_2	0	7	32	72	134	210	289	356	410	448	475	491	498	500	0·95
Stimulus 0·4 sec.	O_2	0	4	28	62	119	186	263	333	393	435	467	485	496	499	1·33
	N_2	0	7	27	65	119	193	237	337	393	436	467	486	495	500	1·26
	N_2'	0	4	27	69	126	197	270	339	395	439	468	487	497	500	0·94
	N_2''	0	4	30	70	128	193	263	329	387	426	462	480	493	499	0·78

It will be seen from the observations recorded above that, whereas the "control" deflections for 0·1 sec. heating differ widely from those for 0·1 sec. stimulus in oxygen or in nitrogen—showing that the heat-production when the live muscle is stimulated is not "instantaneous" as is that in the control observations—the deflections given by the muscle in oxygen are practically identical with those in nitrogen for all three durations of stimulus and up to 5 secs. following excitation. The average difference between the oxygen and the nitrogen deflections is only 3 on the reduced scale of maximum deflection = 500 which corresponds to only about 0·25 mm. on the original records. It is noticeable that this very small average difference is always in the same direction, viz. that the nitrogen deflection always increases definitely though very slightly more rapidly to its maximum than does the oxygen deflection. This small difference is doubtless due to the oxidative recovery processes which, although they go on very slowly at 0° C. must have at any rate some small effect in the first few seconds. Apart from this very small difference however, amounting on the average to 0·6 p.c. of the whole quantity observed, we may assert with fair certainty that the initial processes of heat-production following excitation are independent in character of the presence or absence of oxygen, so that the chemical breakdowns or changes following immediately on excitation are entirely non-oxidative in nature. The rôle of oxygen in muscular activity is displayed simply in the later stages, viz. those of oxidative recovery from previous activity, and not at all in the initial stages. The same conclusion has been reached from a variety of previous experimental work, *e.g.* that of Weizsäcker(4): it seems however to be established, more or less finally by the above, and other similar, experiments.

The experiment shows also that the character of the initial heat-production is uninfluenced by a moderate degree of activity, even in the absence of oxygen. Thus successive breakdowns of the muscle substance, occurring in successive contractions, follow the same time-course so far at any rate as the liberation of energy is concerned and so long as undue fatigue is avoided.

The next important point brought out is that at low temperatures, such as 0° C., where the processes of contraction and relaxation are very much slowed, definite differences in shape are shown between the records given by the heat-production of live muscles and those given by the warming of dead ones. In Fig. 7 are shown three curves taken directly from three records made on the same muscles and plotted on the same scale so as to show the same maximum deflection. It will be seen that B, given by stimulation of the live muscle for 0·1 sec. differs considerably from C, given by warming the same muscle after death for 0·1 sec. It should be noted in estimating the relative certainty of the observed difference that each curve is read to within about 0·2 p.c. of its maximum deflection and that successive records of the same control heating usually do not differ from one another by an average quantity of more than 0·3 p.c. of the maximum deflection. The fact that B rises more slowly than C can be explained only by the fact that heat is produced by the live muscle considerably after the stimulation is over, and the analysis of curve C carried out as described above, and shown graphically in Fig. 9, proves that such indeed is the case. The rate of heat-production is rapid at the start, falls quickly to zero and remains at zero for nearly a second until, at or about the end of relaxation, there is suddenly a considerable quantity of heat evolved. In A, Fig. 7, is shown also a record given by the same muscle stimulated for 1·2 sec., and in Fig. 9 is given the result of an analysis of curve A; it is seen that the observed record for 1·2 sec. stimulus differs from that for 0·1 sec. stimulus by about as much as the latter differs from the control curve given by 0·1 sec. warming: while the analysis as before shows a diminishing rate of heat-production as the stimulus proceeds, followed by a gap, and followed again by a large evolution of heat during or immediately after relaxation.

The same thing is well shown by the experiment of which the analysed results are given in Fig. 10. This experiment was also carried out at 0° C. and a series of stimuli of durations increasing from 0·1 sec.

to 2·5 secs. and diminishing again to 0·1 sec. was given in order. By taking the mean of the two records for any one duration of stimulus the effects of fatigue were largely eliminated from the comparison of the different durations. It is seen from this figure that:

(*a*) a large amount of heat is liberated in the initial 0·2 sec. at all durations of the stimulus;

(*b*) the heat-production diminishes in rate as the stimulus proceeds until it reaches a steady constant value in the longer stimuli, while falling to zero in the shorter ones;

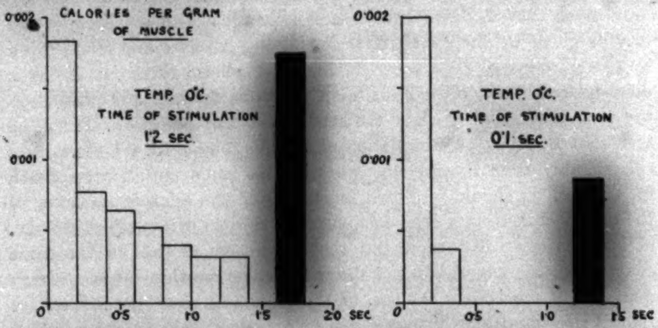

Fig. 9. Analysis of curves in Fig. 7. It will be seen that in the short stimulus (0·1 sec.), there is a large and sudden evolution of heat falling rapidly to zero, followed after an interval of 0·8 sec. by another sudden evolution of heat. These represent the first and third phases of the heat-production, viz. those associated with contraction and relaxation. In the longer stimulus (1·2 sec.) there is an initial large evolution of heat of approximately the same size as before, settling down however to a constant value and falling to zero when the stimulus ceases. After a shorter interval there is again a large and sudden evolution of heat. These represent the first three phases, viz. the heat-production associated respectively with the development, the maintenance, and the disappearance of the mechanical response. Heat-productions given in absolute units of heat.

(*c*) in every case the heat-production associated with the contraction is followed, after an interval, by another burst of heat-production, which it is natural to associate with the relaxation;

(*d*) the interval between the heat-production of contraction and that of relaxation is considerably diminished as the duration of the stimulus is increased; and

(*e*) the amount of heat associated with relaxation increases with increasing duration of stimulus tending to reach a limit and then to remain constant; but it should be remarked that for temperature 0° C. and for periods of stimulation longer than about 2 seconds this relaxation heat has usually showed signs of diminution; in these cases, however, the process of analysis carried out over such a length of time is not so accurate as in the case of the shorter stimuli and, further, the relaxation heat is in such cases a smaller proportion of the total heat-production and so is more difficult to determine. It is natural to associate this diminution, if it occurs, with fatigue.

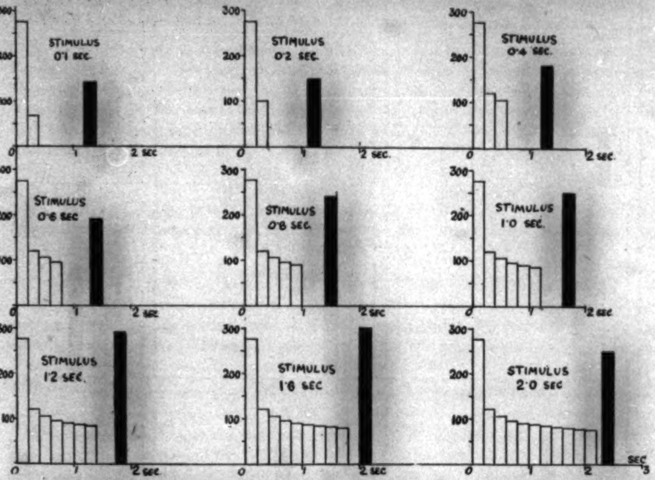

Fig. 10. Exp. B. Heat liberated by sartorius muscles at 0° C. in oxygen. The height of each rectangle represents the heat given out in the interval corresponding to the base on which it stands. Skeleton rectangles represent the heat liberated during contraction, black rectangles the heat liberated during or immediately after relaxation. A rectangle of height 100 denotes a liberation of 0·00066 calories per gram of muscle. Note (*a*) the rapid decline in the rate of heat-production as the stimulus proceeds, (*b*) that the heat-production associated with relaxation increases up to a maximum with increased duration of stimulus, and then slowly declines, and (*c*) that the interval between the heat produced in contraction and that associated with relaxation decreases rapidly as the duration increases. As in Fig. 9 we see the first three phases of the heat-production, the initial high rate, the constant rate attained, and the sudden outburst after the end of the stimulus.

The meaning of these phenomena will be discussed later. A description of further experiments on the same lines follows. In all experiments the stimuli were maximal.

Analysis of the curves recording the rise of temperature of a stimulated muscle.

A. Exp. 1. At 0° C. (*a*) in nitrogen, (*b*) in oxygen. Stimuli given 0·1, 0·2, 0·5, 0·8 and 1·2 sec. Curves identical for N_2 and O_2 up to maximum deflection. Analysis of curves, eliminating fatigue by taking the mean of two records in a series with increasing and then decreasing durations of stimulus.

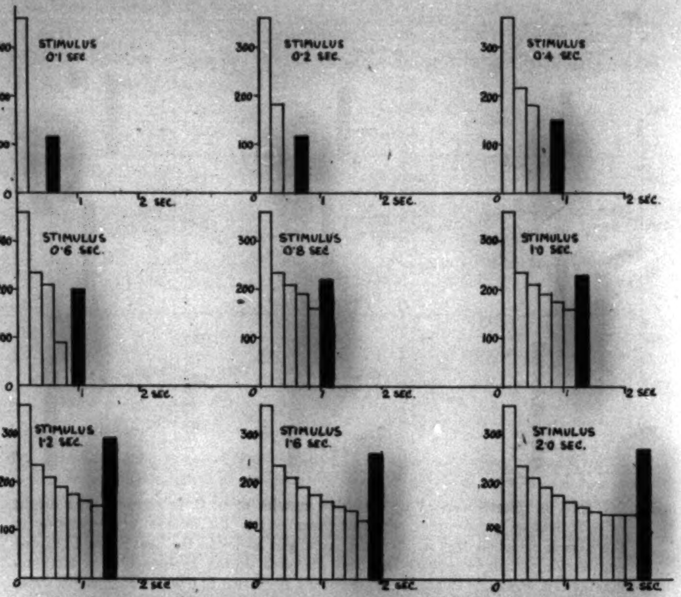

Fig. 11. Exp. C. Heat liberated by sartorius muscles at 10° C. in oxygen. Heat-production in arbitrary units. For description and remarks see Fig. 10. Note also (*a*) that the interval before the third phase of the heat-production is much smaller than at 0° C. (Fig. 11), in fact only about ¼ of it, and (*b*) that compared with the heat liberated in the initial stages the heat associated with relaxation is definitely smaller at the higher temperature.

Time of stimulus, secs.		0·1	0·2	0·5	0·8	1·2
Heat associated with relaxation	proportion of whole	0·26	0·26	0·31	0·34	0·35
	absolute amount*	130	160	260	350	440

* in arbitrary units.

The recovery heat-production in oxygen was very slow being inappreciable in rate after 1 minute.

B. Exp. 2. At 0° C. in oxygen. Fatigue eliminated as above. Results shown in Fig. 10.

Time of stimulus: secs.		0·1	0·2	0·4	0·6	0·8	1·0	1·2	1·6	2·0	2·5
Total heat-production*		495	530	670	750	890	950	1005	1250	1390	1510
Heat associated with relaxation	proportion of whole	0·29	0·28	0·26	0·25	0·27	0·27	0·29	0·24	0·18	0·12
	absolute amount*	140	150	180	190	240	250	290	300	250	180
Interval: secs.		1·1	0·9	0·8	0·7	0·6	0·6	0·5	0·4	0·3	?

* The unit is such that 500 corresponds to 0·0033 calories per gram of muscle. The interval referred to is that between the productions of heat associated with contraction and relaxation.

C. Exp. 3. At 10° C. in oxygen. Fatigue eliminated as above. Results shown in Fig. 11. Note that at this temperature the interval between the end of the stimulus and the moment when the heat associated with relaxation is given off is much less than at 0° C., in fact about ⅓. In the case of the stimuli of longer duration this interval is 0·2 sec. or less. Note also that compared with the heat liberated in the initial stages that associated with relaxation is smaller than at 0° C.

Time of stimulus: secs.		0·1	0·2	0·4	0·6	0·8	1·0	1·2	1·6	2·0
Total heat-production*		480	640	920	1090	1350	1530	1700	1990	2270
Heat associated with relaxation	proportion of whole	0·24	0·18	0·16	0·18	0·16	0·15	0·17	0·13	0·12
	absolute amount*	115	115	150	200	220	230	290	260	270
Interval: secs.		0·4	0·4	0·4	0·3	0·2	0·2	0·2	0·2	0·2

* In arbitrary units.

D. In several experiments carried out at about 20° C. there was little evidence of the heat-production associated with relaxation. The much greater quickness of the physiological processes of contraction and relaxation makes the analysis practically incapable of discriminating between the two separate portions of heat.

E. In two experiments a muscle was cooled to − 2·8° C. and − 2·0° C. respectively. It was found that for short stimuli (0·5 sec. and less) the curves were practically identical with those corresponding to the same stimuli for the same muscles when at 0° C., showing that the relative amount of the relaxation heat and its position in time were not appreciably altered by the further small lowering of temperature. Only short stimuli were given as it was desired not to fatigue the muscle unduly before reducing it to even lower temperatures.

When the temperature was reduced to − 4·8° C. and − 4·0° C. in the above two cases respectively the muscle froze quite stiff. In the first case it may be worth putting on record that the muscle chamber was opened and the muscle examined when frozen; it was then thawed in Ringer's solution at 0° C., after having been frozen for probably at least half-an-hour, and the chamber was closed and replaced in the flask now at 0° C. preparatory to taking the control curves. After it had remained for about an hour for the temperature to settle down it was found that the muscle had revived and several further curves were taken on giving stimuli; its condition however appeared to have undergone a very considerable change compared with its former state at 0° C., in that (1) two to three times the former maximal stimulus was required before the maximal was now reached; (2) the total heat-production (for the same duration of stimulus) was only about one-half the former total heat-production; (3) the relaxation heat as a proportion of the total heat-production was about one-half the corresponding proportion in the former case, and thus the actual relaxation heat was only about one quarter of the previous relaxation heat; in other words, the muscle behaved as if it had been very much fatigued. The difference from the former state might be considerably less after a longer interval as it was found that a stimulus given immediately after the thawing produced no observable contraction, so that it was supposed at the time that the muscle was dead; thus there was probably some process of recovery going on, which was not complete.

These experiments confirm the conclusions suggested above, which may be summarised as follows:

(*a*) Heat is liberated during the development and maintenance of the contraction.

(*b*) Heat is liberated during, or immediately after, the relaxation.

(*c*) This latter heat increases in absolute amount as the duration of the stimulus is increased at least up to about 1½ seconds at 0° C.

(*d*) The interval between the occurrence of the heat associated with contraction and that associated with relaxation diminishes rapidly as the temperature rises, until at about 20° C. it is practically impossible to measure it. A rise of temperature of 10° C. reduces this interval to about a third. It is considerably smaller for long stimuli than for short ones.

(*e*) The heat associated with contraction and that associated with relaxation are unaffected in their relations by the presence or absence of oxygen, provided fatigue be avoided.

A few experiments were made to test the effects of fatigue on the form of the heat-production. It is found in general that the onset of fatigue induced by severe stimulation causes a progressive diminution of the relative amount of the heat-production associated with relaxation as well as in the total heat-production; thus the absolute amount of the relaxation heat is very much diminished. There also appears to be a further delay in the appearance of the relaxation heat, as shown by the "interval" in the subjoined table, this having the same meaning as in the previous table.

F. Exp. At 0° C. in oxygen. Time of stimulus 0·5 sec.

No. of record		29	30	31	33	36
Total heat-production*		915	730	640	570	385
Heat associated with relaxation	proportion of whole	0·35	0·29	0·28	0·24	0·22
	absolute amount*	320	210	180	155	85
Interval		0·8	0·9	1·0	1·1	1·1

Between records No. 29 and No. 30, 1 min. tetanus was given. Between records No. 30 and No. 31, 1 min. tetanus. Between records No. 31 and No. 33, 1 min. tetanus; 5 min. interval; 1 min. tetanus. Between records No. 33 and No. 36, 3 min. tetanus in periods of ¼ min. tetanus separated by ¼ min. rest; 5 minutes interval; then the same repeated twice.

G. Exp. At 10° C. in oxygen.

No. of record		61	65	70	76
Total heat-production*		1710	1160	970	775
Heat associated with relaxation	proportion of whole	0·23	0·20	0·13	0·11
	absolute amount*	400	230	125	85

* In arbitrary units.

Between records No. 61 and No. 65, 1 sec. tetanus followed by 1 min. rest was given four times. Between records No. 65 and No. 70 the same stimulation was given five times and between records No. 70 and No. 76 the same stimulation was given six times. The "interval" referred to above was about 0·2 sec. when the muscle was fresh, but this became more nearly 0·3 sec. or 0·4 sec. towards the end of the experiment.

Comparison with the mechanical response.

The time-relations of the production of heat in muscular contraction should clearly be compared with the time-relations of the development of tension. In order to eliminate any possible instrumental error in the determination of the curve of tension, especially at the higher temperatures where the mechanical response is very rapid, a special tension lever was constructed to record photographically. This instrument consisted merely of an appropriately shaped portion of a hack-saw blade, soldered firmly at its ends to the two ends of a semi-circular brass rod, carrying a mirror in front and an arm about 8 mm. long behind. The muscles used (a pair of sartorius muscles of frog) were attached by an inextensible wire to the end of the arm and a rise of tension in them caused a slight twist in the hack-saw spring: this was recorded by the deflection produced in a spot of light reflected from the mirror on to a strip of bromide paper carried by a revolving drum. The deflections of the spot of light were proportional to the tensions developed and the magnification of movement was so high (about 500 times) that no appreciable shortening occurred in the muscle: the largest record, which is readable to 0·1 mm., was only 2·5 cm. high, so that the actual shortening of the muscle was about $\frac{1}{20}$ mm. as a maximum. The contractions therefore may be considered to be rigidly isometric. Moreover the frequency of vibration of the device is very high—many hundreds per second—so that it is capable of following even the most rapid contraction with complete accuracy and without superimposed vibrations.

The following experiment was performed with the tension-recording device, and the results are shown in Fig. 12 A and B.

Temperatures 0° C., 13° C., 25·5° C.

At each temperature the muscles were given the following maximal stimuli: (*a*) single shock; (*b*) 0·05 sec. tetanus at 90 periods per sec.; (*c*) 0·10 sec. tetanus; (*d*) 0·20 sec. tetanus; (*e*) 0·40 sec. tetanus; (*f*) 0·80 sec. tetanus; and (*g*) 1·60 sec. tetanus.

From these curves we see clearly (*a*) the great effect of temperature on the rate of rise and fall of tension; (*b*) the great influence at the highest temperature, and the noticeable influence at the middle temperature, of the duration of stimulus. Increasing the stimulus from a single shock to even $\frac{1}{20}$ sec. tetanus has prolonged and increased the development of tension, very considerably at 25·5° C., noticeably at 13° C. and only inappreciably at 0° C.

For our immediate purpose the most important point exhibited by

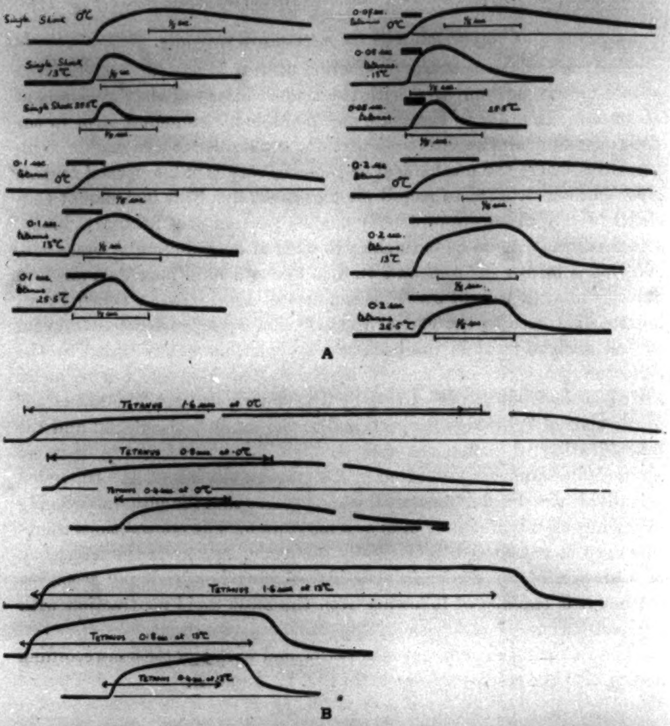

Fig. 12 A and B. Photographic records of the rise of tension in a muscle excited isometrically, at various temperatures (0° C., 13° C. and 25·5° C.), and with various durations of stimulus, special precautions being taken to avoid lag, vibrations, or appreciable shortening. The duration of the stimulus is drawn on each record and in A also the movement in ½ sec.

these curves is the length of the interval between the end of the stimulus and the relaxation. It is necessary to employ some means of comparing the rates of relaxation, and we have used for this purpose the interval between the end of stimulation and the moment when the tension has fallen to half its maximum value. The following table gives the results in the form required.

Table calculated from the curves of Fig. 12.

	Interval between the end of stimulus and "middle" of relaxation: secs.		
	0° C.	13° C.	25·5° C.
Single shock ...	0·57	0·14	0·08
0·05 sec. tetanus	0·51	0·15	0·07
0·10	0·50	0·14	0·07
0·20	0·57	0·13	0·08
0·40	0·45	0·12	—
0·80	0·49	0·11	—
1·60	0·49	0·12	—
Mean...	0·52	0·13	0·075

The results are very consistent. The interval between the end of stimulus and the "middle" of relaxation (defined as the moment at which the tension has fallen to half its maximum value) appears to be a very constant quantity at each temperature, and independent of the duration of the stimulus. The mean values of this quantity are, for the particular pair of muscles used,

at 0° C., 0·52 sec.; at 13° C., 0·13 sec.; at 25·5° C., 0·075 sec.

A rough calculation from these shows that a rise of temperature of 10° C. increases the rate of relaxation about 2·2 times. It is obvious therefore that, if relaxation be accompanied by an evolution of heat, which we desire to separate in our analysis from the heat produced in contraction, it is advisable to work at as low a temperature as possible. At 25·5° C. the "middle" of relaxation occurs only 0·075 sec. after the end of the stimulus; this interval we should expect to be quite inappreciable by our methods of observation and analysis of the heat-production. At 13° C. the "middle" of relaxation occurs only 0·13 sec. after the end of the stimulus; this interval we should expect to be only just appreciable. At 0° C. however the "middle" of relaxation occurs over half a second late and we should expect this quantity to be comparatively easily detected by our methods.

It is noticeable also that, in the short stimuli at 0° C., the interval between the end of the stimulus and the appearance of the main bulk of the heat associated with relaxation lies round and about 0·7 sec., a quantity not far different from the 0·52 sec. between the end of the stimulus and the "middle" of relaxation.

If the experiment on heat-production recorded above be examined it will be seen that the absolute quantity of heat associated with relaxation increases as the duration of the stimulus increases until, at a certain limiting duration, it becomes more or less constant. Throughout this investigation we have imagined that this heat-production is a direct and quantitative expression of the amount of potential energy lost during the relaxation of the muscle. If this be so we should expect that the potential energy, *i.e.* the maximum work the muscle is capable of performing, would increase as the duration of the stimulus increased, becoming constant at about the same limiting duration. We have therefore performed experiments with the inertia mechanism described in another paper (5) in order to determine the effect on the maximum work of varying the duration of the stimulus. It will be seen from the following account that the maximum work increases as the duration of the stimulus increases up to a certain limit of the duration, and then remains constant. At higher temperatures the constant value is reached at a shorter duration of the stimulus. The maximum work seems to reach its constant value more quickly than does the heat-production associated with relaxation, but, in general, the two phenomena are governed by much the same relations and it would appear reasonable to conclude that the potential energy and the heat of relaxation are really one and the same thing.

The experiments on the maximum work are given in the following table.

Effect of duration of stimulus on the maximum work.

Exp. (1). Sartorius muscles 3·4 cm. long, weight 0·33 gram. In Ringer's solution. Maximal stimuli, single phase alternating current at 90 ~. "Equivalent mass" (Mk^2/a^2) of inertia system as used 1600 grams. The arrows denote that the observations were made in the order indicated: the mean of the two observations at any one duration is taken to avoid the effects of fatigue.

A. At 1·5° C. Mass of rider 3 grams. Initial tension on muscles 10 grams weight.

Duration of stimulus: secs.	0·1	0·2	0·3	0·4	0·6	0·8
Work done: gr. cm. →	22	28½	33	35	35	34½
←	22	27	30	32	33	—
Mean work done	22	28	31½	33½	34	34½

A′. At 1·5° C. Mass of rider 5 grams. Initial tension on muscles 17 grams weight.

Duration of stimulus: secs.	0·1	0·2	0·3	0·4	0·6	0·8
Work done: gr. cm. →	23	28	30½	32	34	34½
←	22½	26	28	30	33½	—
Mean work done	23	27	29	31	34	34½

B. At 12° C. Mass of rider 5 grams. Initial tension on muscles 17 grams weight.

Duration of stimulus: secs.	0·1	0·2	0·3	0·4	0·6
Work done: gr. cm. →	36	59	72	72	69½
←	32½	49½	61	65	—
Mean work done	34	54	66½	68½	69½

C. At 21° C.: as A′ and B.

Duration of stimulus: secs.	0·1	0·2	0·3	0·4	0·6
Work done: gr. cm. →	48½	91	101	101	94
←	38	72	86½	89	—
Mean work done	43	81½	94	95	94

D. At 29° C.: as A′, B and C.

Duration of stimulus: secs.	0·1	0·2	0·3	0·4	0·5
Work done: gr. cm. →	46	84	93	90	85
←	20	43	68	76	—
Mean work done	33	63	80	83	85

This experiment shows

(a) that the maximum work in a *short tetanic contraction* increases rapidly as the temperature increases, at any rate from 1·5° to 21° C.;

(b) that the maximum work increases as the duration of the stimulus increases, up to the following limits: 1·5° C. 0·6 sec.; 12° C. 0·4 sec.; 21° C. 0·3 sec.

Beyond these limits the maximum work is unaffected by a further duration of the stimulus.

Exp. (2). Sartorius muscles, about 0·25 gr. wt. and 3·3 cm. long. In Ringer's solution. Maximal stimuli. All observations made with increasing and decreasing duration, as in Exp. (1), and the mean taken for each duration, so as to eliminate fatigue.

A. 8° C. Equivalent mass $Mk^2/a^2=900$ grams. Rider 3 grams. Initial tension 10 grams wt.

Duration: secs.	0·1	0·2	0·4	0·6	0·8
Mean work: gr. cm. ...	19½	30	36	37	37

A′. 8° C. As A. Rider 5 grams. Initial tension 17 grams wt.

Duration: secs.	0·1	0·2	0·4	0·6	0·8
Mean work: gr. cm. ...	16	21	37	39	39

A″. 8° C. Equivalent mass 1600 grams. Rider 3 grams. Initial tension 10 grams wt.

Duration: secs. ...	0·1	0·2	0·3	0·4	0·6	0·8
Mean work: gr. cm. ...	15	19	24	27½	28	28½

B. 1·5° C. As A″.

Duration: secs. ...	0·1	0·2	0·3	0·4	0·6	0·8	1·0	1·2
Mean work: gr. cm. ...	10½	12½	13	13½	15	15½	15½	15½

B′. 1·5° C. As A″ but Rider 2·1 grams. Initial tension 7 grams wt.

Duration: secs.	0·1	0·2	0·4	0·6	0·8	1·0
Mean work: gr. cm. ...	10	10½	12	12½	12½	12½

C. 8·0° C. As B′.

Duration: secs.	0·1	0·2	0·3	0·4	0·6	0·8
Mean work: gr. cm. ...	13½	17	19½	21	21½	22

D. 16° C. As A″.

Duration: secs.	0·1	0·2	0·3	0·4	0·6	0·8
Mean work: gr. cm. ...	16	28	37	42	43	43½

E. 24° C. As A″.

Duration: secs.	0·1	0·2	0·3	0·4	0·5	0·6
Mean work: gr. cm. ...	15	36	55	61	63	63

From these observations it is seen that in spite of oncoming fatigue the maximum work in a tetanic contraction increases rapidly as the temperature is raised (see especially

B', C, D and E). Further the work increases with increasing duration of stimulus up to a certain limit of duration.

The increase of maximum work with increased duration of stimulus is similar therefore in its general character to the increase of delayed heat-production. It would be very difficult experimentally to make the comparison a directly quantitative one, upon the same muscles at the same time: the general agreement however between the ways in which the maximum work and the delayed production of heat increase with increased duration of stimulus, is additional evidence that the potential energy of the excited muscle is the immediate parent of the production of heat which occurs during relaxation.

3. THE RECOVERY HEAT-PRODUCTION.

We have described above an experimental investigation of the first three phases of the heat-production in muscles. It remains to discuss the fourth phase, that of recovery. It was shown in a previous paper (1) that in the slow process of recovery after activity considerable heat is evolved. This heat is presumably "waste heat," evolved in the chemical processes of recharging the muscle to its previous condition: a large part of the energy liberated in the oxidative recovery processes does not presumably appear as heat at all, but is used in restoring the muscle to its previous condition, being stored in some form inside it. We should expect however that the heat produced in recovery would run parallel to the actual process of recovery. We have performed a few experiments to determine the course of the recovery heat-production by our later and better methods. A full account of the subject will be given in a later paper: here we will merely describe the general results in so far as they concern the subject under discussion.

The first point of interest which has appeared is that in the experiments in oxygen at 0° C., we have never succeeded in estimating the recovery heat. The curve of galvanometer deflection for the live muscle stimulated for 0·1 sec. is so very close to the control curve (corresponding to warming for 0·1 sec.) after the first 20 seconds or so that the *rate* of heat-production during recovery must be very small in this case. But whenever we have made an experiment in oxygen at a higher temperature, *e.g.* at 20° C., under otherwise identical conditions, the recovery heat-production has come out quite clearly. It would seem that one or other of the following conclusions is inevitable: (*a*) the recovery heat-production goes on exceedingly slowly at low temperatures; or (*b*) the recovery heat-production is very small at low temperatures. The first alternative seems the more likely, though the second is not impossible. We will consider (*b*) first and then return to the consequences of (*a*). It is not impossible that recovery should take place with a high "efficiency" and that nearly all the energy of the oxidations occurring in recovery should be stored as free energy ready for the next contraction, very little of it appearing as heat. Preliminary experiments have shown that at 20° C. the recovery process goes on with about 58 p.c. "efficiency," heat represented by 72 being evolved in a process in which energy represented by 100 is stored. A temperature of 20° C. however is rather high for an English frog, and occurs only at seasons when food is easy to obtain. It might easily be the case that at low temperatures the processes of recovery are much more economical, until at 0° C. nearly all the energy obtained from oxidations is stored as free energy ready for a subsequent contraction.

It is impossible however to be sure that the real explanation of the experimental facts is not merely that recovery goes on so slowly at low temperatures that it is impossible to detect it. If recovery be quickened three or four times by a rise of 10° C., the rate of heat-production at 0° C. would be only about one-tenth of that at 20° C. and such a slowing down would explain the impossibility of detecting much heat in those three or four minutes subsequent to stimulation during which alone the investigation is reliable. It might be possible to quicken up the rate of recovery at 0° C. by using a high pressure of oxygen, in which case if the recovery heat-production becomes detectible it will be possible to decide between the two alternatives.

If it prove to be the case that recovery from activity is much slowed by a fall of temperature, it is a very pertinent fact in the life of cold-blooded animals. A very distinct limit would be put to their activity at low temperatures, and we have a further sign of the advantage of a high body temperature in recovery from fatigue. Such a high temperature-coefficient is in keeping with what is known of the effects of temperature on most of the chemical reactions occurring in the body, and on the whole it appears to be the more probable alternative.

At higher temperatures we have found it possible to determine also the course of the heat-production in point of time.

In two experiments at 20° C. in oxygen it was found that after a rapid outburst of heat during the development and disappearance of the mechanical response the rate of heat-production rose for about 40 seconds, reached a maximum and fell slowly to zero again until (presumably) recovery was complete. The fall to zero might have been expected; the gradual increase up to the maximum however is more difficult to understand: we should rather have expected the recovery process to start at a high level and to fall continually to zero. The only plausible explanation seems to us at present to be that the processes of recovery are complex in nature, and that preliminary chemical reactions involving only a relatively small production of heat have to precede the final oxidative changes yielding the main bulk of the energy. We may add that some experiments on muscles from which oxygen had been carefully excluded showed signs of a smaller prolonged heat-production during the three minutes subsequent to excitation: this would be in keeping with the hypothesis that non-oxidative changes precede the oxidative breakdowns associated with recovery.

We have avoided going into the subject more in detail here as the investigations now proceeding may throw considerably more light on this interesting side of muscular activity.

4. Discussion.

It is clear that much more work requires to be done before even the general nature of muscular contraction is certain. One obvious way in which our knowledge can be improved is by conducting experiments, on the lines indicated here, upon a variety of other tissues and muscles: another is to amplify these experiments by an investigation of the effects of salts and drugs, or of the presence or absence of other chemical bodies, upon the extent and time-relations of the four phases of the heat-production of muscles. The most prolific step however would be to develop some chemical, or physico-chemical method, capable of following the production or decay of some constituent of the muscle (*e.g.* the hydrogen ion), and of exposing its time-relations as accurately as the thermopile and the tension-lever expose those of the production of energy. At present, apart from the mechanical and electrical responses, the production of heat can be followed and recorded with incomparably greater accuracy and speed than can any other change accompanying muscular activity. An investigation of the heat-production therefore will enable us to sketch the general lines, but it is, and must remain, quite incapable of filling in the details of the picture: the laws of thermodynamics "deal with events, not with the mechanism of events" (6).

Many theories of muscular contraction have been put forward, but not so many of muscular relaxation: in view of the fact that relaxation has been placed, by these experiments, on the same plane as contraction as a process in which active interchanges of energy take place, one is tempted to speculate on its nature. The extent to which a rise of temperature increases its rate would lead one to suspect chemical rather than physical changes as the basis of relaxation. The constancy, for a given temperature, of the interval between the end of the stimulus and the "middle" of relaxation, and its independence of the duration of the stimulus, would suggest that in this interval we have some fundamental and measurable characteristic of the relaxation process. The most important point however appears to be that the process of relaxation is, under all conditions, an irreversible one in the thermodynamic sense: *i.e.* the mechanical potential energy produced in an isometric contraction is not reabsorbed by the muscle as free energy but is allowed to degenerate into heat by some irreversible process unknown. So much indeed is clear: it seems to us however to be wiser to leave the matter there for the present rather than to indulge in detailed speculations for which experiments give us, as yet, no warrant. In order to make the position hitherto attained in regard to the muscle the more intelligible we have included next a description of a physical system exhibiting precisely the same general relations as the muscle in the matter of exchanges of energy. The reader will see at once that this is not a theory of contraction, but merely a physical analogy to what has already been proved in regard to the muscle and its energy relations. The analogy is a very close one, and a close analogy may be valuable in suggesting lines on which further progress can be made, as well as in showing that a certain type of mechanism is possible.

5. An electromagnetic analogy.

Consider an electromagnet attracting a piece of soft iron. To make the magnet exert this attraction a current has to be passed and energy has to be expended in magnetising the electromagnet; to maintain the attraction it is necessary to maintain the current and the expenditure of energy. On breaking the current in the electromagnet the attraction disappears, while the magnetic energy of the electromagnet and of the magnetic field vanishes as such and reappears as heat caused by induced currents. Suppose further that the current was obtained from an accumulator: in order to recharge this accumulator it is necessary to employ some kind of engine to turn a dynamo and in this recharging process heat is liberated in the engine and "free" electric energy is stored in the accumulator.

Store of free energy capable of being liberated without oxidative changes.

The accumulator can have its energy released merely by pressing a key and making an electric contact, just as the muscle can have its energy released by giving it a shock. The chemical changes liberating the energy of the accumulator do not involve any oxidative breakdowns, just as the initial processes of contraction in the muscle do not. The energy of the accumulator may be liberated largely as mechanical potential energy or work, or on the other hand if the accumulator be short-circuited it may be wasted as heat: the muscle's internal energy similarly may appear as potential energy or work or it may as a result of various conditions, *e.g.* in rigor or as a result of treatment with ethyl-alcohol (Weizsäcker(4)), appear entirely as heat.

Trigger action for release of energy. The amount of energy required to make an electric contact and so to start the discharge of the accumulator is very small, as is the amount of energy required to stimulate a muscle.

Development and maintenance of mechanical response. Directly the key is pressed down the current starts in the coil of the electromagnet, and the magnetic attraction develops. The current however does not immediately rise to its full value, owing to the self-induction of the coil nor does the soft iron of the magnet become magnetised instantaneously under the influence of the magnetic field of the coil: hence the attraction exerted by the magnet on the piece of iron increases gradually up to a certain limit and then remains constant: these phenomena are similar to those shown by a stimulated muscle. Moreover if the current be left on too long the accumulator becomes polarised, the current falls off and the magnetic field decreases: while if the accumulator be given a period of rest it recovers and provides its full E.M.F. again, and the magnetic field rises to its previous value. The phenomena of muscular fatigue are analogous.

Disappearance of mechanical response. On opening the electric circuit the magnetic field of the electromagnet diminishes rapidly though not instantaneously to zero, and the pull on the piece of iron falls off. This process is analogous to relaxation.

Performance of work. The current in the electromagnet develops a store of magnetic potential energy in the soft iron core and in the field round it. This magnetic energy will do external mechanical work, if allowed, by pulling up the piece of soft iron to the magnet. On the other hand no external mechanical work is done if the piece of iron be held fast. In the same way the muscle develops elastic potential energy on excitation: this energy may result in external mechanical work if the muscle be allowed to shorten, but in an isometric contraction no external work is done. The work actually performed depends, in both cases, on the "conditions of loading."

Heat liberated in the development of the mechanical response. The energy of the current passing through the electromagnet reappears in two separate forms: (*a*) in the magnetic potential energy of the field of the electromagnet, and (*b*) in Joule's heat liberated in the coils. If the current passes only for a very short time the main part of the energy appears first in the "free"' form (*a*); while if it passes for a longer time an increasing fraction of it appears in the "bound" form (*b*). Similarly with the muscle, energy is liberated (*a*) as mechanical potential energy and (*b*) as heat, (*a*) being the greater in a short response and (*b*) the greater in a long one.

Heat-production in the maintenance of the mechanical response. In order to maintain a constant magnetic field a constant current has to be maintained in the coils of the electromagnet, the whole of the energy of which is degraded into heat. This is analogous to a tetanic contraction.

Heat produced in the disappearance of the mechanical response. When the electromotive force in the circuit of the electromagnet is removed the potential energy of the magnetic field disappears in the process of inducing a current in the coil of the electromagnet or in neighbouring conductors, the energy of this current being dissipated finally as heat. Thus when we remove the E.M.F. exciting the electromagnet there is not a cessation of the heat-production; the heat-production continues until the whole of the magnetic energy of the field has been dissipated as heat through the intermediation of induced currents. Similarly in the muscle, as shown in this paper, when the stimulus ends the heat-production does not stop at once: it continues until all the elastic potential energy of the muscle has disappeared.

The recovery process. The whole of the phenomena described above are independent of the immediate presence of oxygen. When however it is required to recharge the accumulator an engine is started up, oxygen and fuel are consumed, heat and mechanical energy are liberated, a dynamo is turned and current is produced. Similarly in the muscle, when recovery is necessary some mechanism removing lactic acid is set in motion, oxygen is consumed, heat is produced and the muscle is restored to its previous condition.

The heat produced at rest. It is known that accumulators left standing "run down" gradually of themselves. This process of "running down" must liberate heat, and if the accumulators are to be kept in condition it is necessary to recharge them. This continual recharging also involves a production of heat. Similarly the muscle even when kept in oxygen-free atmosphere at rest liberates heat (together with lactic acid) continuously for long periods: if sufficient oxygen be present the running down process is balanced by a continual recharging one with an increased production of heat.

The maintenance of tension in an unstriated muscle. Some unstriated muscles can, unlike the striated ones, exert a considerable tension for long periods without any appreciable increase in the CO_2 output. This fits into our analogy if we suppose that the core of the electromagnet is made of hard steel instead of soft iron, so that the magnetic pull is maintained with no further expenditure of energy until a current is sent into the electromagnet in the opposite direction to demagnetise it.

SUMMARY.

1. Instruments and methods are described by which the heat-production of a muscle can be recorded photographically, if necessary to less than 0·000001° C. These methods have the advantages: (*a*) it is possible to work at any temperature desired; (*b*) the muscle can be immersed in any required gas or solution during the observations; (*c*) the zero, once attained, is relatively very stable; (*d*) no errors arise from differences of temperature along the muscle or thermopile; (*e*) the mechanical response therefore can be recorded simultaneously with the heat-production, in any manner desired; (*f*) the response to the heat-production is relatively rapid; (*g*) exact "control curves" can be made by electrical heating; and (*h*) calibration in absolute units of heat is accurate and easy.

2. A numerical method of analysing the photographic records is described. By means of this the time-relations of the heat-production can be investigated, not only in the slow processes of recovery, but in the more rapid processes immediately following excitation.

3. By working at 0° C. on the sartorius of the frog it is shown that the *time-relations* of the heat-production in the first few seconds after excitation are independent of the presence of oxygen. Weizsäcker showed that the *magnitude* of this initial heat-production is unaffected by oxygen. Hence we may regard the initial chemical breakdown following excitation as being entirely non-oxidative in character.

4. In a prolonged contraction (*e.g.* 2 secs. tetanus) of the sartorius,

the heat-production may be resolved into the following four phases: (*a*) an initial rapid production, diminishing gradually in rate as the stimulus proceeds; (*b*) a smaller constant heat-production maintained so long as the stimulus is maintained, and ending shortly after the stimulus ceases; (*c*) a relatively large evolution of heat, occurring rather suddenly during the later stages of relaxation; and (*d*) a large, but slow, production of heat occurring in the presence of oxygen for some minutes after the contraction is over.

In the twitch evoked by a single shock or by a very short tetanus only three of these phases occur, (*a*), (*c*) and (*d*). It is natural to associate (*a*) with the development, (*b*) with the maintenance and (*c*) with the disappearance of the mechanical response, and to connect (*d*) with the processes of recovery. It is probable that the heat produced during relaxation is derived from the mechanical potential energy developed on excitation, and lost in relaxation.

5. The interval between the second and third phases of the initial heat-production depends largely on the temperature. In a short twitch at 0° C. it is of the order of 0·7 sec.: at 25° C. it is too short to be measured directly with the instruments at present employed. All the more reliable experiments have been performed at a low temperature.

6. Photographic records are given of the course of the mechanical response in an isometric contraction, at various temperatures and for various durations of stimulus. The instrument used had a high frequency of vibration, a high magnification, and inextensible connections to the muscle. It was found that, provided fatigue be avoided, the interval between the end of excitation and the "middle" of relaxation is independent of the duration of the stimulus, but is increased two or three times by a fall of temperature of 10° C. This effect of temperature is similar to its effect on the interval between the second and third stages of the heat-production.

7. The absolute amount of heat liberated in relaxation increases as the duration of the stimulus increases, up to a certain limit, after which it remains more or less constant.

8. By employing an inertia system for measuring maximum work, it is found that in a short tetanic excitation: (*a*) the maximum work increases as the duration of the excitation increases, up to a certain limit, after which it remains constant; and (*b*) the maximum work in a tetanic stimulus of given duration is considerably increased by a rise of temperature.

It is concluded from (7) and from 8 (*a*) that the maximum work, and the heat-production of relaxation, are derived from the same thing, the potential energy liberated on excitation.

9. The methods described have made it possible to follow the course of the evolution of heat in the fourth phase of contraction—recovery—and preliminary experiments at 20° C. have shown that the heat-production of recovery increases in rate for 30 or 40 secs., and then falls gradually to zero. It is suggested that the oxidative processes of recovery are complex, the initial stages involving non-oxidative reactions associated with a smaller evolution of heat, the later stages involving the more energetic oxidative changes.

10. At low temperatures such as 0° C. the recovery heat-production in oxygen is almost inappreciable by the methods employed. It is concluded either: (*a*) that the processes of recovery go on very slowly at low temperatures, being subject to a temperature coefficient of the same size as most chemical reactions occurring in the body; or (*b*) that the processes of recovery at a low temperature are much more "efficient" than at a high one, nearly the whole energy of the oxidised bodies being stored in the muscle ready for a subsequent contraction. Of these alternatives the first seems the more probable.

11. A close analogy to the energy relations of a muscle and the four phases of its contraction is provided by those of an electromagnet excited by a current from an accumulator.

The expenses of this research have been borne in part by a grant from the Royal Society to one of us (A. V. H.).

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THE PARTITION OF CO_2 BETWEEN PLASMA AND CORPUSCLES IN OXYGENATED AND REDUCED BLOOD[1].

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IT has been shown by Zuntz(1), Alexander Schmidt(2), and Frédéricq(3)[2], that the serum of blood contains more CO_2 than the corpuscles. On the other hand, it is stated that if CO_2 acts on the serum and corpuscles which have been separated from one another, then the corpuscles contain more CO_2 than the serum. This has been explained by an interchange of acid and basic radicles between corpuscles and serum which is considered to occur naturally in the blood. Such an interchange is supported by Hamburger's experiments(4), and also confirmed by those of Van Slyke and Cullen(5), which showed that treating blood with CO_2 caused the plasma to lose chlorine which must have entered the corpuscles. Quite recently de Boer(6) has shown that there is also a loss of sulphate from the plasma under these conditions.

In the present research the attempt has been made to study systematically the partition of CO_2 between corpuscles and plasma at different CO_2 pressures in human blood.

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[1] The expenses of this investigation were defrayed by a grant from the Royal Society.

[2] See also Loewy's account in Oppenheimer's *Hdb. d. Bioch.* (Jena 1911), 4, Pt. I, p. 64.

In their well-known paper **Christiansen, Douglas and Haldane**(7), showed that at pressures of CO_2 up to 110 mm. reduced blood took up more CO_2 than oxygenated blood and they explained this by assuming that reduced hæmoglobin was a weaker acid than oxyhæmoglobin. We have tried to carry the results one stage further, by finding out how this increase of CO_2 in reduced blood is distributed as between corpuscles and plasma.

Method. The method employed has already been briefly described by us(8). Human blood was exposed in tonometers to varying pressures of CO_2, being rotated in a water bath at 38° C. for 15 minutes. The blood was delivered beneath liquid paraffin in two portions. One was used for the estimation of the CO_2 in the whole blood by Van Slyke's apparatus(9). The other portion was centrifugalised and the CO_2 of the plasma also estimated. Towards the end of this work a few determinations of the CO_2 in the corpuscular mixture were made.

Some more details may be given. A separate tonometer was used for each experiment. The tonometers had a capacity of 350 to 400 c.c. The lower part was drawn out so as to hold a cylindrical column of 6 c.c. blood. The analysis of the gas was carried out in duplicate after the blood had been delivered and when the tonometer had cooled to the temperature of the room. The calculation of the partial pressure was carried out as described by **Barcroft**(10). A correction was introduced for the amount of blood originally in the tonometer. Thus if the tonometer had a volume of 350 c.c. and contained 6 c.c. of blood when in the water bath, the volume of gases in the tonometer should be taken as 344 c.c. It must be admitted that the tonometer method, as far as calculating the CO_2 pressures is concerned, is more involved than the method described by **Parsons**(11) or by **Christiansen, Douglas and Haldane**(7). Its advantages are that duplicate analyses can be readily performed. Further the preliminary preparation of the mixed gases is a very simple affair, as it is only necessary to add the requisite measured quantity of CO_2 from a burette. It was quite easy to arrange that the final pressure in the apparatus should be about the same (*i.e.* 10 to 20 mm. in excess of atmospheric pressure) so that the corrections applied worked out to be about the same in each case[1].

In all the determinations with Van Slyke's apparatus, after obtaining the first reading of mixed CO_2, oxygen and nitrogen, the CO_2 was absorbed by caustic soda introduced from the cup of the apparatus, and a second reading taken. A second extraction was then carried out and the CO_2 again absorbed. Very occasionally a third extraction was required. We are indebted to Mr Parsons for pointing out a possible source of error in our method. After delivery of the blood beneath liquid paraffin the sample cooled to the temperature of the room. It was possible that this in itself might cause an alteration of the distribution of CO_2 between plasma and corpuscles. This was tested by putting an electric centrifuge into an incubator kept at 38° C. There was also another centrifuge at room temperature. The following results were obtained for the serum of oxygenated blood which had been exposed to a pressure of 74·5 mm. CO_2.

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[1] It has recently been found that the calculated pressure agrees satisfactorily with the actual pressure in the bath measured by a manometer.

Exp.	CO_2 in 100 c.c. plasma, c.c.	Remarks
1	68·36	Sample centrifuged at once, at room temperature (20° C.)
2	69·1	Sample transferred at once to incubator for 5 minutes and then centrifuged
3	69·3	Sample centrifuged at room temperature after 1 hour in ice
4	66·7	Sample centrifuged in the incubator after resting 1 hour in the incubator

In Exp. 4 there is some loss of CO_2 owing presumably to the length of time the sample was kept at 38° C. and the increased diffusion through paraffin at this temperature. The other experiments show that lowering temperature before centrifuging makes no perceptible difference. In Exp. 2 the object of keeping the sample for five minutes in the incubator is to allow it to regain its original temperature, because after delivering the sample into the centrifuge tube the latter had to be counterpoised at room temperature. The whole process only took about one minute but a little cooling must have occurred.

Another experiment with partially reduced blood gave similar results. The CO_2 pressure was 41·7 mm. and the oxygen pressure 35·3 mm.

CO_2 in 100 c.c. plasma, c.c.	
58·4	Centrifuged at once at 18° C.
59·5	Centrifuged after standing at 18° C. for 1 hour

In carrying out the experiments with reduced blood, the reduction was carried out in two stages. The blood was first shaken in a vessel of about 800 c.c. capacity, which had been exhausted by an air-pump two or three times, and filled with nitrogen from a cylinder. Some CO_2 was also added. The shaking took place at room temperature for about half an hour. The tonometers were also filled with nitrogen and CO_2 in the same way. The blood was transferred from the large vessel to the tonometers without coming into contact with air. The nitrogen in the cylinder contained a little oxygen so that the blood was never completely reduced. This was thought to be an advantage, because it was possible that the highly unphysiological condition of complete reduction might cause an alteration in the properties of the hæmoglobin. The oxygen pressure was always determined by a single analysis of the tonometer after the experiment.

The relation between the log K of Hill's (12) formula, and the CO_2 pressure of J. J.'s partially oxygenated blood was determined (see Table I)[1] and was found to agree quite well with the curve for Barcroft's blood, investigated by Barcroft and Poulton (13), and with Debenham and Poulton's results (14). From the O_2 pressure it is a simple matter to calculate the percentage saturation of the hæmoglobin with oxygen by Hill's formula, using the value for log K corresponding to the particular CO_2 pressure. This has been done (see Table VII, col. 8) for J. J.'s defibrinated blood; the values are less than 20 p.c. with two exceptions. In one the per cent. saturation works out at 22·4 p.c., and in the other the actual value found by Van Slyke's modified method (15) was 23 p.c. The mean value for all these percentage saturations was found to be 11 p.c. so that the curves, to be later described, may be said to represent the average CO_2 dissociation curve of J. J.'s defibrinated blood containing about 10 p.c. of its hæmoglobin saturated with oxygen. In the case of W. R. and J. J.'s oxalated blood, it can be seen from the oxygen pressures that the variation in the percentage saturation are not as great as in J. J.'s defibrinated blood.

In nearly all the determinations when the CO_2 in the blood was being estimated a drop of the same blood was taken for a hæmatocrit determination. Fine bore glass tubes were made. The blood was run into the tube and one end was sealed in a flame. The tube was rotated for 15 minutes in a high speed disc centrifuge. Very often the hæmatocrit determinations were carried out in duplicate.

Oxygenated and reduced blood.

Chief attention was paid to J. J.'s defibrinated blood. The results for the CO_2 per 100 c.c. blood, and per 100 c.c. serum, separated as described, after equilibrium between blood and CO_2 at 38° C. had been established are given in Table VII and plotted out in Fig. 1. From these results two curves have been drawn, one for blood (B), another for what may be described as "True Serum" (T.S.). It will be noticed that up to 90 mm. CO_2 pressure the blood curve lies below the "true serum" curve. Table II (cols. 2 and 4) gives the numerical values of these curves at certain stated pressures. The mean of all the hæmatocrit determinations shows that up to 90 mm. CO_2 100 vols. of blood contained 50·93 vols. of corpuscles and 49·07 vols. of serum[1]. Using these values the various results shown in Table II are calculated. The CO_2 content of the serum in 100 c.c. blood is first calculated (col. 6). This subtracted from the CO_2 content of the whole blood (col. 2) gives the CO_2 content of the corpuscles in 100 c.c. blood (col. 9). These calculated results are represented by curve C in Fig. 1.

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[1] The tables are given at the end of the paper.

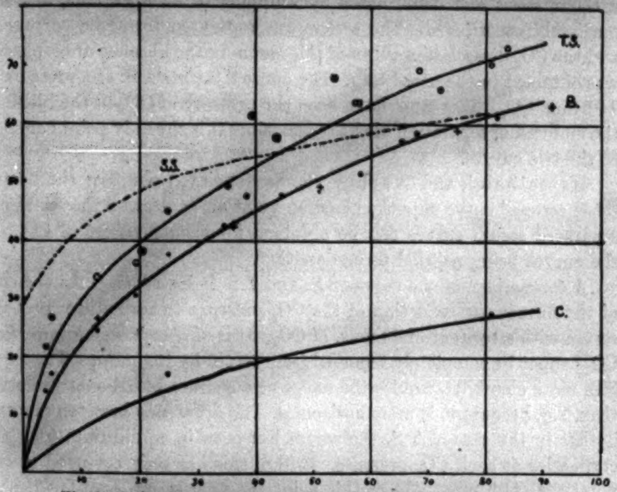

Fig. 1. Ordinate, percentage vol. CO_2. Abscissa, CO_2 pressure, mm.
● Defibrinated blood. Corpuscles of 100 c.c. blood.
+ Oxalated blood.
○ True serum from defibrinated blood.
⊕ True plasma from oxalated blood.
S.S. Curve for separated serum.

Attention is particularly directed to the behaviour of the CO_2 in serum and corpuscles, as shown respectively by the areas between

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[1] **Hamburger** has stated that treating corpuscles with CO_2 increases their volume. We have not been able to observe in our hæmatocrit determinations any evidence of this up to 90 mm. pressure of CO_2 although in the comparatively few measurements made at higher pressures we thought that there probably was a small increase in volume. It has seemed best to take the mean of all hæmatocrit observations up to 90 mm. in order to construct the curves in Fig. 1.

B and C, and between C and the base line. At 0 mm. pressure there is no CO_2 in either. This corresponds to the well-known fact that all the CO_2 of blood can be withdrawn by the pump. As the pressure of CO_2 increases, there is a gradual rise in the CO_2 content of both; but the serum always contains more CO_2 than the corpuscles.

In Fig. 1 a fourth curve S.S. is also drawn (see also Table III). This represents the "Separated Serum." Blood was withdrawn from a vein without stasis and defibrinated by whipping in an open dish, and immediately centrifuged. The serum was separated from the corpuscles and the CO_2 dissociation curve of this serum in the absence of corpuscles was obtained in the usual way. The curve S.S. cuts T.S. at a pressure of 44·5 mm. CO_2. This must have been the pressure of CO_2 in the blood at the moment of separation of the serum, since it is the only point common to the two curves.

Hasselbalch and Warburg(16) have pointed out that the "separated serum" curve cuts the blood curve at an angle, and that different separated serum curves can be obtained for each pressure of CO_2, all the curves being parallel to one another.

A comparison of the curves S.S. and T.S. is instructive. In the case of the former the reduction of the CO_2 pressure to zero still leaves the serum with a content of 29 vols. of CO_2. It is of course well known that CO_2 cannot be completely removed from serum by the pump. The curve S.S. more closely resembles the curve of a sodium bicarbonate solution than T.S. because it is more horizontal. The difference between the two is that in the case of T.S. the serum has been in equilibrium with the corpuscles at each CO_2 pressure. In S.S. this has only occurred at one point viz. 44·5 mm. Above this point the true serum contained more CO_2 than the separated serum, and below, less CO_2. This difference of behaviour can only be explained by an interchange of acid and basic radicles between serum and corpuscles and Hamburger's(5a) and de Boer's work(6) points to a migration of Cl and SO_4 ions into the corpuscles with rise of CO_2 pressure.

Now it is quite obvious that a steady migration of these ions alone would be impossible as the first ones would form a layer of charged particles inside the corpuscles which would prevent the migration of others similarly charged. Hence this migration must be accompanied by a corresponding migration of hydrogen ions or of OH ions in the opposite direction. This would cause an increase in the hydrogen ion concentration of the corpuscles at the expense of the serum. De Boer has explained the migration of SO_4 ions as a complicated mechanism to help in the excretion of sulphates in the urine. While not denying that this may be one effect of the interchange, the chief reason for its occurrence would seem to be much simpler. It is to prevent the corpuscles from becoming rapidly more alkaline than the serum and the same applies to the migration of Cl ions.

It is possible to infer from Hasselbalch and Warburg's experiments that the CO_2 dissociation curve of separated red corpuscles is steeper than the blood curve and very much steeper than the separated serum curve. The diffusion of acid that takes place from the serum into the corpuscles with rise of CO_2 pressure by making the corpuscles more acid lessens the steepness of the curve.

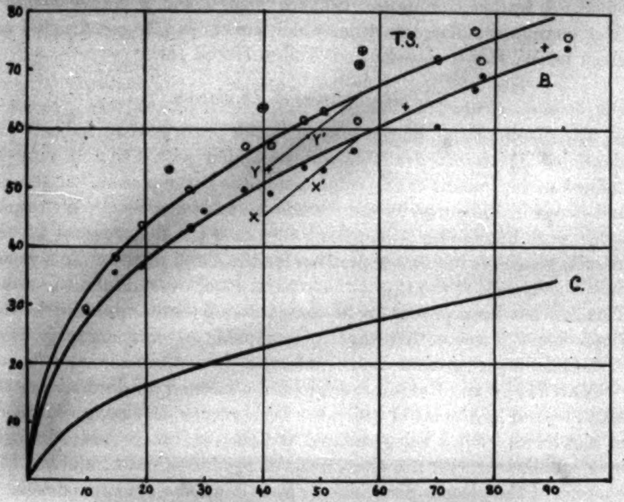

Fig. 2. Ordinate, CO_2 vol. Abscissa, CO_2 pressure, mm.

● Defibrinated blood. ○ True serum.

+ Oxalated blood. ⊕ True plasma.

Of course a transference of base from corpuscles to serum would be as effective as the migration of acid just described, and de Boer has argued from Hamburger's work that the corpuscles are permeable to metallic ions. It is quite possible the two processes occur simultaneously although the evidence for the latter process is not so strong as for the former.

Similar calculations have been made for J. J.'s reduced defibrinated blood. The corresponding curves are shown in Fig. 2. It will be noticed that the reduced true serum curve bears a similar relation to the blood curve, and although the separated serum was not investigated there can be no doubt that it would be similar in its behaviour to the oxygenated separated serum. Consequently we must assume that migration of ions takes place between serum and corpuscles with alteration of CO_2 pressure similar to that which we have shown to occur in oxygenated blood. A further comparison between Figs. 1 and 2 is deferred to a later section. Similar data from which curves can be constructed are given for W. R.'s oxalated blood (Tables II and III).

The alkali reserve of the plasma.

The alkali reserve of the plasma is the term used to indicate the alkali in the plasma available for combining with CO_2. It may be defined as the amount of CO_2 combined in the "true plasma" at 40 mm. and it can be measured by the method we have described. We cannot agree with Stadie and Van Slyke(17) that the CO_2 content of the arterial plasma is the best expression for the alkali reserve. This would only be the case if the C_H of the arterial blood was always the same. This has not been proved to be the case and results obtained in this Department indicate that the C_H is capable of comparatively wide variations in pathological conditions.

Van Slyke and Cullen(5) originated a method for determining the alkali reserve of the blood which has been very widely adopted. Blood is withdrawn from a vein, oxalated and the separated plasma brought into equilibrium with the alveolar air of the investigator, and the CO_2 content of the plasma determined. Now it will be obvious on looking at our curves that for any given case the value so obtained will depend on the CO_2 pressure in the original blood as withdrawn, because there is a different separated blood plasma curve for each CO_2 pressure. The only occasion on which an absolutely accurate result would be obtained would be if the pressure of CO_2 in the blood before centrifuging happened to be 40 mm. exactly. The farther the pressure is removed from this point, the more inaccurate the result will be. This point is very well illustrated by a series of results published quite recently by Stadie and Van Slyke(17). They determined the CO_2 content of the centrifuged plasma from arterial and venous blood. They also determined the CO_2 capacities of the same samples (*i.e.* the CO_2 content of the separated plasma at 40 mm. according to Van Slyke and Cullen's method).

The first point of interest is that the difference between the arterial and venous CO_2 content is very variable. This is after all to be expected because the difference will depend on the rate of metabolism in the hand and on the rapidity of the blood flow. We do not agree with the authors that the variability can be explained by errors of experiment. The second point of interest is that where the difference between the CO_2 content is large, the difference between the CO_2 capacities is also large and *vice versa*. The following table has been compiled from 27 of their experiments where the data are complete.

No. of Exps.	Average difference between venous and arterial CO_2 contents	Average difference between CO_2 capacities	Remarks
3	−1·3	−0·47	Arterial blood plasma contained more CO_2 than venous
5	0·52	0·58	Difference between contents less than 1 %
4	1·88	2·08	Difference between 1 and 2 %
15	4·87	2·92	Difference greater than 2 %

It cannot be supposed that the differences between the capacities is caused by alteration in the amount of bases or non-volatile acid due to circulation through the hand, because if lactic acid were formed the venous capacity would be diminished whereas in the great majority of cases it was increased. It can readily be explained by our results, since the greater the difference between the CO_2 content of the sample of blood the greater will be the difference between the corresponding separated plasma curves. No doubt Van Slyke and Cullen's method is capable of detecting large variations in the alkali reserve, but owing to the error just described we are doubtful if it is capable of detecting the slighter and yet abnormal variations which are often present in pathological conditions.

Van Slyke, Stillman and Cullen(18) give the normal limits of the alkali reserve as 53 and 78 vols. p.c. Now by calculation from Hasselbalch's formula mentioned in the next section, the p_H's corresponding to these limits are 7·33 and 7·48 respectively. It is difficult to believe that the limits for the p_H of normal individuals are really as wide as this. Donegan and Parsons(19) in a few cases found values varying from 7·36 to 7·41. At present it is impossible to dogmatise, but the errors inherent in Van Slyke and Cullen's procedure may partially account for these wide limits.

Van Slyke and Cullen realised that there was an interchange of ions between corpuscles and plasma with change of CO_2 pressure and they pointed out the necessity for preventing escape of CO_2 between the time when the blood was withdrawn from the arm, and its being centrifuged. However in the original method described, some escape of CO_2 probably did take place, as the blood was not delivered beneath paraffin. Our results have shown what a serious error would be caused if any appreciable amount of CO_2 was allowed to escape and we think this error has probably vitiated some of the results published by other authors, since a loss of CO_2 would cause an apparent lowering of the alkali reserve and would lead to the conclusion that an acidosis was present, which was really not the case.

Stadie and Van Slyke(17) have introduced an alternative method of determining the alkali reserve, viz. measuring the CO_2 content of the venous plasma, the blood being centrifuged beneath albolene. We think that this method is also open to grave objections particularly owing to the variations in the CO_2 content of the venous blood which has already been mentioned.

The calculation of the C_H *of blood.*

The importance of the "true serum" CO_2 dissociation curve lies in the fact that the hydrogen ion concentration of defibrinated blood can be calculated from it.

Hasselbalch(20) has stated that assuming[1] that the CO_2 in blood is present as bicarbonate, the blood proteins acting solely as acids, the H ion concentration can be calculated by the following equation.

$$p_H = pK_1 + \log \frac{(\text{Bic})}{(CO_2)}, \text{ where } pK_1 \text{ is a constant,}$$

which can be obtained from **Hasselbalch's** determinations of the hydrogen ion concentration of pure sodium bicarbonate solutions; and (Bic) and (CO_2) represent the molecular concentrations of combined and free CO_2 in the solutions under examination. Now blood is a mixture of two liquid phases—plasma and corpuscles—separated from one another by a semipermeable membrane, and our present experiments show that CO_2 is present in both these phases in different concentrations. Further the solubility of CO_2 in the plasma and corpuscles is also different. Hence it is hardly legitimate, as **Hasselbalch** has done, to calculate the C_H of whole blood by a formula which should really only apply to a single phase or solution.

Parsons(11) has found as the result of measuring the C_H of the "true serum" obtained from oxygenated and reduced blood, that the values for p_H are slightly larger (more alkaline) than those calculated from the CO_2 content of the whole blood by Hasselbalch's[1] equation. These results are shown in the lower part of Table IV, cols. 2–5. He has also found that the p_H of completely reduced defibrinated whole blood is the same as that of the "true serum" using our definition of the term. In other words, when the p_H of reduced blood is measured, what is really measured is the p_H of the "true serum" or "true plasma."

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[1] The succeeding paper by **Campbell and Poulton** shows that this assumption is justified

We wish to state the following proposition—that at a given pressure of CO_2 the p_H of any sample of blood oxygenated or reduced, may be obtained by separating off the serum or plasma without loss of CO_2, measuring its CO_2 content and applying Hasselbalch's formula. In support of this proposition there is the parallelism between the CO_2 dissociation curve of "true serum" and blood which we have found (Figs. 1 and 2). Further we have calculated in the upper part of Table IV (1) (cols. 2 and 4) the hydrogen ion concentration of the serum, which is the same as that of the blood, by applying Hasselbalch's formula to our true serum results, (2) (cols. 3 and 5) the incorrect hydrogen ion concentration of the blood by applying the formula, as Hasselbalch does, to our results for whole blood. These calculations show that the p_H's for the true serum are always somewhat higher than those calculated from the blood, which agrees with what Parsons found.

But the final argument in favour of our proposition, is the very close approximation of the differences between our calculated p_H's for the true serum of J. J.'s oxygenated and reduced blood, to those actually observed by Parsons for his blood. These differences are shown in col. 6. The mean is the same in each case, viz. 0·033.

From the physiological point of view it is of interest to notice that at 40 mm. the p_H of J. J.'s blood (*i.e.* "true serum") is 7·32 when oxygenated and 7·35 when reduced. It is perceptibly more acid than Parsons' blood but only very slightly more acid than some cases that Donegan and Parsons have examined. In subject H. of their paper the p_H for the reduced blood was 7·36. In the case of W. R. the p_H's of the oxygenated and reduced blood at 40 mm. were 7·35 and 7·37 respectively.

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[1] It must be admitted that in Hasselbalch's paper there is excellent agreement between his two sets of figures, viz. those calculated from his formula from the CO_2 content of blood, and those actually measured with his hydrogen electrode. This agreement is probably fortuitous because as Parsons has shown his p_H measurements are really too low owing to incomplete reduction of the blood, and his calculated values are incorrect owing to the inapplicability of his formula to a mixture of two liquid phases such as blood.

The CO_2 *dissociation curve of true serum and blood at high* CO_2 *pressures.*

From the theoretical point of view it was of interest to investigate the partition of CO_2 between serum and corpuscles at pressures above 100 mm. J. J.'s oxygenated blood was used in these experiments.

Our best thanks are due to Mr Campbell who kindly gave us some help. The actual determinations are given in Table V. The few hæmatocrit determinations which were made showed that the volume of the corpuscles had a mean value of 53·5 p.c. and this has been used in making the calculations. The actual determinations and the curves are shown in Fig. 3 and in Table V. It is to be specially noted that these determinations and curves represent combined CO_2 and thus differ from the preceding experiments in which the total CO_2 was dealt with. B. and T.S. are dissociation curves giving the combined CO_2 in 100 c.c. blood and true serum and C shows the combined CO_2 in the corpuscles of 100 c.c. blood. Some actual measurements of the CO_2 content of the corpuscular mixture obtained after centrifuging are also shown in the figure. On the whole they agree very well with curve C, which is satisfactory because

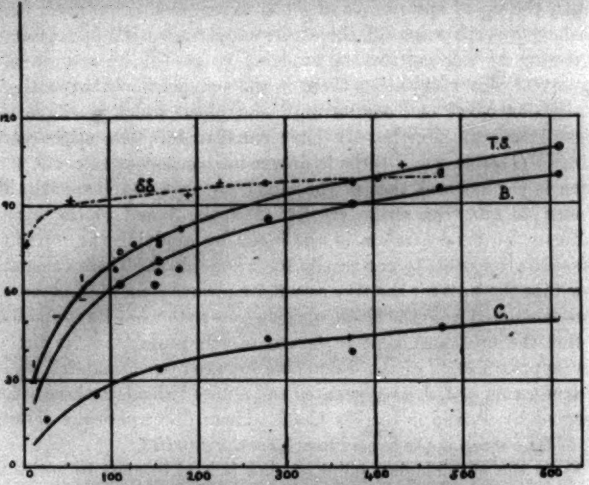

Fig. 3. Ordinate, CO_2 vol. Abscissa, CO_2 pressure, mm.
● Defibrinated blood. Corpuscles in 100 c.c. blood.
○ True serum. + Separated serum.

curve C is obtained by calculation indirectly from curves B and T.S. and this agreement shows that our method of calculation is permissible.

It is noticeable that the true serum curve remains above the blood curve and parallel to it up to 600 mm. CO_2. This means that at these high pressures the true serum still contains more CO_2 than the same volume of corpuscles. We have shown that at pressures below 100 mm. as the CO_2 pressure increases, there is a migration of acid from serum to corpuscles, and the problem presented itself to us whether at these high pressures a similar explanation was also necessary. To find this out we saturated some blood at 404 mm. CO_2, centrifuged it beneath paraffin without loss of CO_2, and determined the dissociation curve of the "separated serum." This is the curve S.S. of Fig. 3. It is clear that this curve cuts T.S. at the point of separation and that below this point the separated serum contains much more CO_2 than the true serum. The same explanation must hold in this case as in the case of the serum separated at 44·5 mm. In other words we must assume a steady migration of acid from serum to corpuscles even when the pressure is raised to ten times the physiological level.

This result is of particular interest in view of the results described in the succeeding paper. There it is shown that at 400 mm. the reaction of both serum and corpuscles is on the acid side of the isoelectric points of their constituent proteins, which means that all the available sodium is present as bicarbonate, and that the proteins are combining directly with CO_2, and acting the part of bases. Even under these conditions on raising the CO_2 pressure in blood, acid still passes from serum to corpuscles. It will probably be found that this interchange which takes place on both sides of the isoelectric point has a simple physicochemical explanation on the basis of the envelopes of the red cells acting as semi-permeable membranes and of hæmoglobin being a stronger acid and stronger base than the serum proteins. However without more experimental evidence on the subject further speculation is unprofitable.

It is of interest to compare the dissociation curve of J. J.'s defibrinated blood with other results already published. Van Slyke and Cullen and Hasselbalch and Warburg, have both published curves for ox blood. The three curves are compared in Table VI. There is fairly good agreement between Hasselbalch and Warburg's results for ox blood and our results for J. J.'s defibrinated blood. The discrepancy between Van Slyke and Cullen's results and those of Hasselbalch and Warburg, both of them for ox blood, would seem to be much too great to be explained as due to normal variation. Since

Hasselbalch and **Warburg's** and our results agree fairly well the presumption is that **Hasselbalch** and **Warburg's** results are the more correct. **Van Slyke** and **Cullen** express their results in "Molecular concentration of chemically bound CO_2 in solution." We have expressed them as percentages, assuming that 0·01 on their scale represents 22·4 c.c. per 100 c.c. of solution.

Straub and **Meier**(21) have stated that at somewhere about the iso-electric point of hæmoglobin the direction of the dissociation curve of blood suddenly alters, because each molecule of hæmoglobin takes up one molecule of CO_2 in direct combination. Their curves appear to us very unconvincing and the changes of direction described well within the error of experiment, especially as they used minute volumes of blood for their determinations. They also took no account of the partition of CO_2 between corpuscles and plasma.

The transport of CO_2 *by the blood in the body.*

So far we have dealt with the amount of CO_2 combined in serum and corpuscles, but this gives no information as to which of these two substances is chiefly concerned with the transport of CO_2 in the body. In the tissues the blood receives CO_2 and gives up oxygen; in the lungs the blood gives up CO_2 and receives oxygen. Does this interchange of CO_2 (*i.e.* the transport of CO_2) take place chiefly in the plasma or chiefly in the corpuscles?

We have attempted to answer this question. The problem is by no means an easy one because the difference in the CO_2 content of the oxygenated and reduced blood is small, varying in our experiments from about 5 to 9 vols. p.c. depending on the pressure of CO_2. Again although he CO_2 of the plasma is determined directly, the CO_2 in the corpuscles is the difference between two measurements, and the errors of experiment are magnified. We have diminished the effect of experimental error as far as possible by making numerous observations. We have further made a few observations on J. J.'s oxalated blood and have examined more fully the oxalated blood and plasma of W. R. a patient suffering from tabes dorsalis. The detailed results are given in Tables VII and VIII. They are summarised in Table II.

In comparing the CO_2 in corpuscles and plasma, it is possible to deal either with the CO_2 in 100 c.c. corpuscles and plasma, or with the corpuscles and plasma in 100 c.c. blood. The latter is the more physiological comparison and is the one adopted here. In the case of J. J.'s defibrinated blood it makes very little difference, since there are nearly equal volumes of corpuscles and plasma in the blood. The "difference columns" in Table II show that there is a gradually increasing difference with rise of CO_2 pressure between the CO_2 in oxygenated and reduced corpuscles (col. 11) and also between the corresponding true serums or true plasmas (col. 8). Taking the sum of these differences for comparison, it is shown that in the case of J. J.'s defibrinated blood, the value for the corpuscles in 100 c.c. blood is 28·4 and for serum 13·6. The former figure is more than double the latter. This is of considerable physiological significance, because it means that on nearly complete reduction of the blood, the corpuscles take up about twice as much CO_2 as the plasma; on oxygenation the process is reversible. The corresponding numbers for W. R. are 15·25 and 10·15, the ratio between these numbers being 1·5. This is less than the previous ratio because the corpuscular volume in 100 c.c. was considerably less than the plasma volume. In cases of anæmia when the corpuscular volume is very much reduced it is clear that actually more CO_2 will be taken up by the plasma than by the corpuscles.

The meaning of these results is clear. Oxyhæmoglobin is a considerably stronger acid than reduced hæmoglobin, so that oxygenation by itself drives out CO_2 from the red corpuscles. If this were the only thing that happened we should expect that if the CO_2 pressure were kept constant the corpuscles alone would show a difference in CO_2 content on oxygenation, the plasma CO_2 remaining constant. This however is not the case. In J. J.'s defibrinated blood at 40 mm. the reduced corpuscles and serum contain respectively 22·4 and 28 p.c. CO_2. The oxygenated corpuscles and serum contain 19·1 and 25·9 p.c. CO_2. Hence oxygenation of the hæmoglobin drives out CO_2 from the serum as well as from the corpuscles but not to such an extent in the former case.

The only possible explanation is that oxygenation causes a diffusion of acid from corpuscles to plasma, or, conversely, a diffusion of base from plasma to corpuscles. The reason for this interchange is clear. If hæmoglobin could diffuse out into the plasma, oxygenation would cause this diffusion to take place, since this process causes an increase in the C_H inside the corpuscles and there would be a tendency to equalise the C_H on each side of the corpuscular envelope. Since hæmoglobin is indiffusible this tendency to the equalisation of the CO_2 is brought about by the diffusion outwards of other acids which are diffusible or the diffusion inwards of diffusible bases.

These considerations point to the extreme complexity of the factors influencing the interchange between corpuscles and plasma actually occurring in the body, when the blood passes through the lungs or enters the tissues. In the latter case the increase in the CO_2 tension causes by itself a diffusion of acid into the corpuscles, as was pointed out previously, while the partial reduction of the hæmoglobin intensifies this process.

It has already been mentioned that the reduced blood in our experiments was on the average about 10 p.c. saturated with oxygen. It would be natural to suppose that as blood gradually becomes oxygenated at constant CO_2 pressure, there is a gradual diminution in the CO_2 content of the corpuscles and plasma throughout the whole extent of the process, but this requires proof because it might be possible that both in our experiments and those of Christiansen, Douglas and Haldane, the loss of CO_2 occurred and was practically complete right at the beginning of the oxygenation and that over the physiological range oxygenation produced no change.

Fortunately we have some experiments, all carried out on the same date, with J. J.'s defibrinated blood which show that the former is the correct interpretation. The results are shown in Fig. 3 by short horizontal lines for the CO_2 of blood and short vertical lines for the CO_2 of the serum. The details are given in Table I. When the oxygen saturation of the blood was 61·1 p.c. the combined CO_2 of the blood and serum was 57·6 and 65·4 c.c. at 67·6 mm. This is shown in the figure by the right-hand short horizontal and vertical lines. They are decidedly above the corresponding curves for fully oxygenated blood and serum. With an oxygen saturation of 42·6 p.c. the left-hand vertical and horizontal lines at 10·9 mm. are placed still higher in the diagram, the value for blood now coinciding with the true serum curve. When the oxygen saturation was reduced to 10 p.c. the blood value at 37·3 mm. was actually 4 vols. above the true serum curve. The serum result was lost in this experiment. Hence we may conclude the CO_2 content of blood and serum increases *pari passu* with the reduction of the hæmoglobin.

It is of interest to compare the observations on J. J.'s oxalated blood from the point of view of the effect of removing fibrinogen on the CO_2 content of the blood. The observations on the oxalated blood and plasma are specially marked with crosses in Figs. 1 and 2. It is seen that oxalated plasma has a distinctly higher CO_2 combining power than the "true serum." On the other hand the difference between the CO_2 combining power of defibrinated and oxalated blood is by no means so obvious. Slightly more CO_2 is combined in reduced oxalated blood than in reduced defibrinated blood, but the difference is hardly outside the experimental error. There is no noticeable difference in the case of the oxygenated blood.

In our preliminary communication (8)[1], we pointed out that the reduction of blood caused the corpuscles to take up about twice as much CO_2 as the plasma, and so we concluded that the transport of CO_2 in the blood was carried out chiefly by the corpuscles. Although this conclusion is in the main correct, there is no doubt that the part played by the corpuscles is exaggerated by the figures in Table II, because in the body the blood is never reduced to anything like 10 p.c. oxygen saturation. In order to try to get a true picture of what actually takes place in the body we have made a similar calculation to that of Christiansen, Douglas and Haldane, an example which Parsons has also followed in calculating the change of p_H in the circulating blood. J. J.'s blood was used for this calculation because the curves are probably more accurate than those of W. R. J. J.'s oxygenated blood at 40 mm. which is taken as the arterial tension, contains 45 c.c. CO_2. This is marked as X in Fig. 2. Similarly the corresponding true serum contains 52·8 c.c. This is marked as Y. For simplicity it is assumed that the arterial blood is fully saturated with oxygen so that X and Y represent the arterial blood at 40 mm. Now the oxygen capacity of J. J.'s blood was 18·7 p.c. Reduction of 100 c.c. blood to 10 p.c. saturation will yield 16·83 c.c. O_2 and in addition practically all the oxygen in solution, viz. 0·17 c.c. in all 17 c.c. If all this oxygen were used up in the body 14·0 CO_2 would be formed, if the R.Q. is 0·82.

On the other hand if the blood were completely reduced in the body the amount of CO_2 formed would be 15·56 c.c. Under physiological conditions blood is never completely reduced in the body. Lundsgaard (22) has shown that the oxygen unsaturation in the venous blood of the arm is a variable quantity but that usually the percentage unsaturation is not greater than 40 p.c. so that the actual increase of CO_2 in the venous blood would be 6·2 c.c. the total volume in the venous blood being 51·2 c.c.

In Fig. 2, curve B corresponds to 10 p.c. oxygen saturation; it has been pointed out that this corresponds to an addition of 14 c.c. CO_2 so that the blood at this saturation would contain 59 c.c. CO_2. On the curve the pressure at this point is 57 mm. and at this pressure the true serum contains 66 c.c. Fine lines have been drawn between these points

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[1] It should be noted that we have since slightly altered the lowest part of the curve for J. J.'s reduced blood.

X and Y and they represent the condition of blood and true serum on gradual reduction to 10 p.c. oxygen saturation.

In the body the venous blood contains 51·2 c.c. and is represented by the point X′. The condition of venous true serum will be Y′, *i.e.* 58·8 c.c. The pressure being 47·7 mm.

The volume of CO_2 in the plasma of 100 c.c. venous blood will be 28·9 c.c. and the CO_2 in the corpuscles will be 22·3 c.c. In the arterial blood the corresponding figures are 25·9 and 19·1. Hence as the blood becomes venous the plasma will take up 3·0 c.c. and the corpuscles 3·2 c.c. which means that for all practical purposes there is very little difference between the corpuscles and plasma as transporters of CO_2.

When this research was started the attempt was made to decide the relative importance of corpuscles and plasma in the transport of CO_2 by analysing arterial and venous blood and the corresponding plasmas. Laidlaw and Poulton carried out a number of observations on cats the blood being received into oxalated cannulas and delivered beneath paraffin into centrifuge tubes. Two observations were also made on human subjects. It became clear however, that the differences found had nearly the same magnitude as the experimental error and so this plan was dropped, and the method just described of determining the complete dissociation curves of oxygenated and reduced blood and plasma was adopted instead.

CONCLUSIONS.

(1) The dissociation curves of oxygenated blood and of blood reduced to 10 p.c. oxygen saturation and the corresponding "true plasma" curves have been determined. At all pressures of CO_2 up to 600 mm. the "true plasma" contains more CO_2 than the corpuscles in equilibrium with it.

(2) As the pressure of CO_2 increases there is a steady migration of acid from the plasma into the corpuscles, so that the dissociation curves of the plasma and the corresponding corpuscles are quite different from the dissociation curves of separated plasma and separated corpuscles.

(3) The C_H of blood whether oxygenated or reduced can be determined by measuring the CO_2 content of the true plasma at a given CO_2 pressure and applying Hasselbalch's formula.

(4) Reduced corpuscles and reduced true plasma contain respectively more CO_2 than oxygenated corpuscles and true plasma, the difference between the corpuscles being greater than the difference between the plasmas; so that if the venous blood were completely reduced, the transport of CO_2 would be carried out chiefly by the corpuscles. However

since venous blood is usually not more than 40 p.c. unsaturated, the transport of CO_2 is carried out by corpuscles and plasma in nearly equal proportions.

(5) On reduction of blood there is a migration of acid from corpuscles to plasma.

(6) The best method of determining the alkali reserve of the plasma is to measure the CO_2 content of the true plasma of oxygenated blood at 40 mm.

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TABLE I. Defibrinated blood (J. J.).

log *K*	CO_2 pressure mm.	Combined CO_2 in 100 c.c. blood c.c.	Combined CO_2 in 100 c.c. "True Serum" c.c.	Per cent. saturation with oxygen
$\bar{4}\cdot99$	10·9	29·4	35·5	42·5
$\bar{4}\cdot45$	37·3	51·8	—	10·0
$\bar{4}\cdot10$	67·6	57·6	65·4	61·1

TABLE II. The partition of CO_2 between plasma and corpuscles in oxygenated and reduced blood.

I. J. J.'s defibrinated blood (mean hæmatocrit value = 50·93).

1	2	3	4	5	6	7	8	9	10	11
	CO_2 in 100 c.c. blood		CO_2 in 100 c.c. {serum plasma		CO_2 in {serum plasma of 100 c.c. blood			CO_2 in corpuscles of 100 c.c. blood		
CO_2 pressure mm.	O_2 Hb. c.c.	Red Hb. c.c.	O_2 Hb. c.c.	Red Hb. c.c.	O_2 Hb. c.c.	Red Hb. c.c.	Difference	O_2 Hb. c.c.	Red Hb. c.c.	Difference
10	22·4	27·0	29·9	32·0	14·7	15·7	1·0	7·7	11·3	3·6
20	31·8	37·3	39·3	43·4	19·3	21·3	2·0	12·5	16·0	3·5
30	39·0	44·5	46·8	50·7	23·0	24·9	1·9	16·0	19·6	3·6
40	45·0	50·4	52·8	57·1	25·9	28·0	2·1	19·1	22·4	3·3
55	52·3	58·1	61·0	65·1	29·9	31·9	2·0	22·4	26·2	3·8
70	58·5	65·0	67·8	72·0	33·3	35·3	2·0	25·2	29·7	4·5
90	64·3	73·0	74·1	79·5	36·4	39·0	2·6	27·9	34·0	6·1
							13·6			28·4

II. J. J.'s oxalated blood (mean hæmatocrit value = 44·5)

40	44·5	52·3	58·0	63·5	32·1	35·2	3·1	12·4	17·1	4·7
55	51·1	60·0	69·0	71·5	38·2	39·6	1·4	12·9	20·4	7·5
							4·5			12·2

III. W. R.'s oxalated blood (mean hæmatocrit value = 39·77).

15	32·0	33·7	37·6	41·8	22·6	25·15	2·55	9·4	8·55	−0·85
25	38·8	44·3	47·5	51·6	28·55	31·05	2·5	10·25	13·25	3·0
35	45·3	52·1	54·4	58·3	32·7	35·1	2·4	12·6	17·0	4·4
45	51·1	56·7	61·2	62·8	36·8	37·8	1·0	14·3	18·9	4·6
60	57·4	60·3	66·2	67·8	39·8	40·8	1·0	17·6	19·5	1·9
75	60·0	62·9	68·8	70·0	41·4	42·1	0·7	18·6	20·8	2·2
							10·15			15·25

TABLE III. "Separated plasma" from J. J.'s defibrinated blood.

CO_2 per 100 c.c. "separated plasma" c.c.	CO_2 pressure mm.	O_2 pressure mm.	Temp. of bath °C.
31·8	1·8	150 approx.	38
43·7	12·2	„	„
48·3	21·1	„	„
53·7	42·0	„	„
59·6	67·2	„	„
63·0	79·0	„	„

All the results in column 2 are the mean of two closely agreeing duplicate analyses

From Fig. 1 it is deduced that the plasma was separated from the corpuscles at a pressure of 44·5 mm. CO

TABLE IV. The calculated C_H of J. J.'s defibrinated blood.

	Oxygenated blood		Reduced blood		
CO_2 pressure mm.	p_H calculated from "true serum"	p_H calculated from blood	p_H calculated from "true serum"	p_H calculated from blood	Difference Cols. 2 and 4
20	7·51	7·45	7·55	7·52	0·04
40	7·32	7·27	7·35	7·32	0·03
50	7·25	7·22	7·28	7·26	0·03
60	7·20	7·16	7·23	7·21	0·03
				Mean	0·033

The C_H of **Parsons'** defibrinated blood.

	p_H "true serum" observed	p_H calculated from blood	p_H "true serum" observed	p_H calculated from blood	
20	7·575	7·52	7·62	7·56	0·045
40	7·38	7·30	7·41	7·34	0·03
50	7·315	7·24	7·34	7·27	0·025
60	7·26	7·20	7·29	7·23	0·03
				Mean	0·033

TABLE V. Defibrinated blood (J. J.). Temp. of bath 38° C.

Date 1919	Combined CO_2 per 100 c.c. blood c.c.	Combined CO_2 per 100 c.c. "true serum" c.c.	Combined CO_2 per 100 c.c. corpuscles c.c.	CO_2 pressure mm.	O_2 pressure mm.	Percentage vol. of corpuscles by hæmatocrit
Sept. 16	—	76·3	—	124·7	150 approx.	52
Oct. 16	—	67·7	—	105·0	„	54·8
„	62·6	—	—	149·0	„	
„	67·1	77·2	—	156·0	„	
„	68·5	81·5	—	180·0	„	
Oct. 23	63·3*	73·8	—	110·3	47·1	52·3
Nov. 28	70·3	76·4	62·5	157·0	150 approx.	53·4
„	90·0	97·5	73·6	376·3	„	53·1
„	100·3	108·9	—	610·0	„	51·5
1920 March 24	84·7	97·2	81·5	278·0	„	—
„	95·8	100·5	88·5	477·0	„	—
„	—†	98·2	—	404·0	„	—
					Mean	53·5
		Combined CO_2 per 100 c.c. "separated serum"				
„	—	76·2	—	3·7	„	—
„	—	91·7	—	55·1	„	—
„	—	97·4	—	226·0	„	—
„	—	103·5	—	430·0	„	—
March 25	—	93·2‡	—	188·0	„	—

* Hb 79 p.c. saturated with O_2.

† 42 c.c. blood used. It was delivered beneath paraffin, and centrifuged. The "separated serum" was used for the subsequent five determinations.

‡ Separated serum kept over night in the cold.

TABLE VI. Combined CO_2 in 100 c.c. oxygenated blood.

CO_2 mm.	J. J. c.c.	Ox blood Hasselbalch and Warburg c.c.	Ox blood Van Slyke and Cullen c.c.
76	54·5	49·0	86·3
152	70·0	64·0	96·0
228	79·3	72·5	103·5
304	85·3	79·0	108·5
380	90·0	84·0	113·0

TABLE VII. Defibrinated blood (J. J.).

Date 1919	Total CO_2 per 100 c.c. blood c.c.	Total CO_2 per 100 c.c. "true serum" c.c.	CO_2 pressure mm.	O_2 pressure mm.	Percentage vol. of corpuscles by hæmatocrit	Temperature of bath °C.	Calculated % saturation with O_2
Aug. 4	30·7	36·1	19·7	150 approx.	48·0	38·0	
"	42·3	49·9	34·6	"	50·5		
"	—	47·7	38·3	"	49·5		
"	47·5	—	50·2	"	50·5		
Sept. 3	24·7	33·5	12·9	"	47·0	37·8	
"	47·8	55·2	44·4	"	50·7	37·5	
"	—	62·8	57·6	"	53·0	37·8	
Sept. 5	14·0	21·7	4·0	"	—	38·0	
"	57·0	—	65·3	"	—	"	
"	—	73·3	83·7	"	—	"	
Sept. 16	17·1	26·7	5·0	"	52·0	"	
"	—	69·5	68·6	"	52·5	"	
"	61·5	—	82·2	"	54·0	"	
Oct. 16	51·5	62·5	58·0	"	54·8	"	
"	—	66·1	72·1	"			
Nov. 27	37·7	45·2	24·9	"	49·6	"	
"	—	70·3	81·0	"	49·9	"	
Nov. 28	58·4	—	67·8	"	52·3	"	
				(Mean)	51·29		
1920							
Aug. 4	43·0	49·3	27·6	8·6	46·5	38·0	7·90
"	49·8	57·1	36·8	8·1	48·5		5·01
"	53·2	62·8	50·5	14·0	49·2		10·20
"	60·3	71·9	70·1	10·0	47·0		3·99
Sept. 3	35·4	38·1	15·0	6·2	50·0	37·8	5·6
"	48·8	57·2	41·7	4·9	47·1	37·8	1·2
"	53·6	61·4	47·0	7·0	52·6	37·8	2·5
Sept. 16	28·7	28·7	9·9	13·0	50·0	38·0	23·0*
"	56·6	61·3	55·7	16·9	48·1	"	19·0
"	69·2	72·0	77·7	19·0	51·6	"	13·6
"	73·8	76·2	92·7	—	51·8	"	—
Oct. 9	36·6	43·0	19·4	12·7	50·0	"	22·4
"	46·2	—	30·9	12·7	58·8	"	17·0
"	—	68·3	53·8	13·5	54·5	37·2	12·0
"	67·3	77·6	76·8	18·0	53·0	"	12·0
				(Mean)	50·58	(Mean)	11·0

* Value obtained experimentally.

TABLE VIII. Oxalated blood (J. J.).

Date 1919	Total CO_2 per 100 c.c. blood c.c.	Total CO_2 per 100 c.c. "true plasma" c.c.	CO_2 pressure mm.	O_2 pressure mm.	Percentage vol. of corpuscles by hæmatocrit	Temperature of bath °C.
June 6	37·2	—	24·8	150 approx.	—	
"	42·6	—	36·0	"	—	
"	58·5	—	75·2	"	—	38·0
"	63·2	—	91·5	"	—	
"	26·4	—	13·1	"	—	
"	48·0	—	50·3	"	—	
July 23	52·8	—	40·9	16·7	45·1	
"	64·2	—	64·3	1·0		
"	73·7	—	88·2	6·2		
"	—	51·4	24·0	7·8		
"	—	54·2				
"	—	63·4	39·8	5·9		
"	—	57·6	43·5	150		
July 30	—	61·2	38·7	"	43·7	
"	—	68·0	53·6	"	—	
"	—	71·3	56·4	1·0	43·2	
"	—	73·5	57·1	—	43·2	
				(Mean)	44·52	
Oxalated blood. Subject W. R. (Tabes, +Wassermann).						
April 1	32·5	46·8	21·3	150 approx.	42·7	37·7
"	46·8	54·7	39·3	"	46·8	"
April 8	58·0	65·8	62·0	"	34·5	37·8
"	49·0	53·7	34·1	"	34·6	"
April 15	—	37·9	16·9	"	37·5	"
"	45·2	—	42·6	"	37·5	37·7
April 29	51·6	63·3	47·8	"	38·3	38·0
"	54·3	65·2				
May 6	61·0	68·4	74·5	"	39·6	37·8
"	58·6	69·1				
May 13	35·8	—	15·9	"	38·1	37·5
"	33·1					
May 22	29·7	35·3	13·0	"	41·6	38·0
April 15	59·9	63·2	54·0	2·7	40·2	38·2
May 13	39·5	—	21·5	9·0	40·6	38·0
"	47·2	55·3	29·9	9·1	39·8	37·5
"	59·3	65·6	44·3	10·5	42·8	38·0
"	60·0	65·5	64·4	8·9	39·8	37·5
May 22	32·2	38·8	12·6	4·2	38·7	38·0
"	50·2	55·3	30·0	4·0	37·5	"
"	60·7	74·2	72·1	3·2	45·3	"
				(Mean)	39·77	

THE RELATION OF OXYHÆMOGLOBIN TO THE CO_2 OF THE BLOOD[1].

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THE relation of the blood proteins to the transport of the CO_2 by the blood is a problem that has been much debated. In the main there are two opposing views on this subject. According to one view the CO_2 is partly present as sodium bicarbonate, and partly united to the proteins by chemical combination or adsorption. According to the other view, the CO_2 is present altogether as alkaline bicarbonate, while the proteins act as acids, and thus compete with the CO_2 for the alkali, the amount of sodium bicarbonate depending on the concentration of the CO_2 according to the law of mass action.

The early history of the controversy (1–4) has been described fully by **Loewy**, who inclines to the first of these two views as representing the actual conditions in the body. However during recent years many workers (7, 8) have adopted the view that most of the CO_2 is present as bicarbonate, the proteins acting as acids. This is largely due to pioneer work of **L. J. Henderson** (5). **Hasselbalch** (6) using improved methods introduced his well-known formula for the hydrogen ion concentration of the blood. This has been considered by **Joffe** and **Poulton** (14).

The theory of amphoteric electrolytes established by **James Walker** (9), follows directly upon the ionic theory and the law of mass

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[1] The expenses of this investigation were defrayed by a Government Grant from the Royal Society.

action, and stands or falls with these two principles. According to the theory their behaviour depends on the hydrogen ion concentration, the substance acting as a base in an acid medium and acting as an acid when the hydrogen ion concentration is diminished. The isoelectric point when the substance is neutral, lies at an intermediate C_H. Michaelis and others(10) have determined the isoelectric points of hæmoglobin and various serum proteins, and in every case they are more acid than the normal reaction of the blood. Hence if these determinations are accepted these proteins must act as acids in the blood, and only act as bases when the C_H is much greater than ever occurs in the body.

There has been a tendency to regard the behaviour of plasma towards acids as identical with that of a solution of sodium bicarbonate. This has been strenuously opposed by Moore(11) as the result of his early work on the "reactivity" of the plasma.

The theory of direct combination of CO_2 with protein and especially with hæmoglobin, has received a fresh impetus from Buckmaster's work(12). He dialysed red blood corpuscles for varying lengths of time, and found that they took up more CO_2 than could be accounted for by simple solution in the mixture. The amount of combined CO_2 increased with the hæmoglobin content of the mixture, and with the tension of CO_2 to which the mixture was exposed. Buckmaster himself was doubtful whether this reaction played a great part in the body, because it was only at relatively high pressures of CO_2 that much CO_2 was actually taken up in combination. However Bayliss(13) in a very recent paper considers that carbon dioxide is carried by hæmoglobin. He also dialysed serum against sodium bicarbonate containing an excess of free CO_2 so as to ensure that there was no normal sodium bicarbonate present, and under these conditions, which resemble the conditions found in the body, he found that no CO_2 was chemically combined with the serum protein. He also states that the serum proteins do not act as acids; but we do not think his experiment (p. 176) is very conclusive, because actually less CO_2 was found in the serum and bicarbonate mixture than in the bicarbonate solution alone. We shall describe a somewhat similar experiment.

Experiments with dialysed red cells.

On the balance of evidence so far presented we inclined to the view that all the CO_2 is present as bicarbonate, the blood protein and particularly hæmoglobin playing the part of acids. It was important to settle this question definitely, because **Joffe** and **Poulton**(14) have shown that **Hasselbalch's** formula can be applied to determine the C_H of blood by determining the CO_2 content of the "true plasma" or "true serum" at a given CO_2 pressure. **Hasselbalch's** formula is only correct if all the CO_2 is present as bicarbonate. After we had carried out most of the experiments in this paper, we noticed a single uncontrolled experiment in one of **Hasselbalch's** papers(6) bearing on the same point. He used washed ox corpuscles dialysed for only three hours, and to this a solution of sodium bicarbonate was added, so that the solution was ·025*N*. He then exposed the solution to three separate pressures of CO_2 and estimated respectively the content of CO_2. At pressures below 90 mm. the solution contained less CO_2 than corresponded to ·025*N* $NaHCO_3$, and the only possible explanation of this phenomenon is that the hæmoglobin acted as acid.

The importance of this question warrants a full investigation, and for this purpose we have used exclusively human blood. In some experiments blood from a case of myocardial degeneration was used. The patient showed some breathlessness but no cyanosis. In other experiments blood came from three normal individuals. The results of the different series agreed well together. The blood was defibrinated and centrifuged; the serum removed and the corpuscles washed with 0·9 p.c. sodium chloride solution. The corpuscles were dialysed against distilled water for 5–6 days. At the end of dialysis there was no perceptible cloud with silver nitrate and nitric acid. This method was very similar to that used by **Buckmaster**. In all the experiments the solution used contained 52 p.c. hæmoglobin, as tested by a specially standardised Haldane's hæmoglobinometer. This corresponded to 10·85 vols. of oxygen per 100 c.c.

Our first experiment (Table I) was undertaken to confirm **Buckmaster's** results. The solution of hæmoglobin in distilled water was exposed to various pressures of CO_2 at 38° C., and the total CO_2 determined by Van Slyke's apparatus, as described by **Joffe** and **Poulton**. **Joffe** and **Poulton** found that in defibrinated blood the red blood corpuscles occupy about 50 p.c. of the total volume. Hence the hæmoglobin value of red corpuscles on Haldane's hæmoglobinometer scale is 200 p.c. We were using a 52 p.c. solution of hæmoglobin. Now the solubility of CO_2 in red corpuscles at 38° C. is 0·45 (**Bohr**(15)), and in distilled water 0·555. The solubility in our solution falls somewhere between these two values; it was calculated as 0·527. The dissolved CO_2 (Table I, col. 3) has been calculated from this coefficient. The CO_2

actually combined with hæmoglobin is shown in col. 4. It varies from 15·8 c.c. p.c. at 74·9 mm. up to 39·6 c.c. at 805 mm. As a control of our method of analysis we have placed lower down in Table I four determinations of the amount of CO_2 taken up by distilled water at 38° C., and we have placed beside them for comparison the amounts calculated from the known solubility of CO_2 in water.

TABLE I. Vol. CO_2 in dialysed hæmoglobin solution at varying CO_2 pressures at 38° C.

CO_2	100 c.c. hæmoglobin solution contain		
	Total CO_2	Dissolved CO_2	Combined CO_2
mm.	c.c.	c.c.	c.c.
74·9	21·0	5·2	15·8
112	28·6	7·8	20·8
329	49·6	22·9	26·7
805	95·6	56·0	39·6

Vol. CO_2 in distilled water at 38° C.

CO_2	CO_2 found	CO_2 calculated from known solubility
mm.	c.c.	c.c.
79·2	8·7	5·8
217·6	21·8	16·0
415·2	27·8	30·3
570·6	43·2	41·7

Parsons(8) has explained **Buckmaster's** results by supposing that during dialysis the solution of corpuscles which originally contained $NaHCO_3$ lost CO_2, so that some sodium hydrate combined with the hæmoglobin. He suggested that on saturating with CO_2 the hæmoglobin would give up its soda again and bicarbonate would be formed. This explanation will not apply to our experiments because the weight of ash found was 0·20 and 0·16 gms. p.c. respectively in the samples of hæmoglobin used. However calculation showed that the hæmoglobin solution contained 0·21 gm. of Fe_2O_3 per 100 c.c. which corresponds pretty well with the actual values of the ash found.

Now we agree with **Buckmaster** that the CO_2 is actually combined with the hæmoglobin and also probably with the protein of the stroma of the corpuscles, but our explanation is that these solutions are so acid that hæmoglobin being an amphoteric electrolyte is acting as a base. A simple calculation will indicate the actual hydrogen ion concentration of a solution of CO_2 in water. The reaction is represented by $H + HCO_3 \rightleftharpoons H_2CO_3$. By the law of mass action $(H)^2 = K\,(CO_2)$ where (H) and (CO_2) are the molecular concentration of the hydrogen ions and dissolved CO_2 and K is the first dissociation constant of carbonic acid, which is $4{\cdot}15\ 10^{-7}$ at 38° C. according to **Milroy**(16). Suppose we calculate (H) for a solution under a pressure of 40 mm. CO_2, which is about the pressure in the arterial blood. Then the CO_2 dissolved is 29·2 c.c. per litre and the molecular concentration is $\frac{29\cdot 2}{22400}$, therefore

$$(H) = \sqrt{4\cdot 15\ 10^{-7} \times \cdot 0013},$$

$$\therefore\quad p_H = -\,4\cdot 62.$$

Solutions of CO_2 at pressures employed by Buckmaster and ourselves will have a still higher C_H about 1000 times as much as blood. It is thus impossible to draw any conclusions as to the state of combination of CO_2 in blood from these experiments.

The reaction of the blood is regulated by the presence of sodium bicarbonate, and so sodium bicarbonate was next added to the hæmoglobin solution of the previous experiment in such strength as to make a 0·041*N* solution. This particular strength was used because it approximately represented the total amount of alkali in the blood which according to Parsons(8) is available for combining with CO_2. Our control experiment (Table II) consisted in preparing a sodium bicarbonate solution of similar strength in distilled water and estimating its CO_2 content at different pressures. The pressures varied from 1 mm. to 583 mm. The total CO_2 varied from 90·3 to 129·7 c.c. per 100 c.c. of solution; but on subtracting the CO_2 in solution the combined CO_2 was the same at each pressure within the limits of experimental error, the mean value being 91·3 c.c. The fact that the combined CO_2 of a pure sodium bicarbonate solution is independent of the pressure, if the latter is above a millimeter or two, is of course a well-known fact and Buckmaster(17) among others has published similar results.

TABLE II. Vol. CO_2 in $NaHCO_3$ solution at varying CO_2 pressures.

	100 c.c. $NaHCO_3$ solution contain		
CO_2 mm.	Total CO_2 c.c.	Dissolved CO_2 c.c.	Combined CO_2 c.c.
1	90·3	0·1	90·2
15·6	92·0	1·1	90·9
32·5	93·8	2·4	91·4
33·7	91·6	2·5	89·1
62·8	98·2	4·6	93·6
148	103·8	10·8	93·0
221	111·5	16·1	95·4
368	118·0	26·9	91·1
583	129·7	42·6	87·1
			Mean = 91·3

The value 91·3 vols. p.c. may be taken as representing the total available alkali in our solution of hæmoglobin and sodium bicarbonate. Table III gives our results for total and combined CO_2 in this solution at pressures varying from 4·8 mm. up to 596·4 mm. The results for the combined CO$_2$ are plotted out in Fig. 1. The interrupted line represents the CO$_2$ in the corresponding pure sodium bicarbonate solution.

TABLE III. Vol. CO$_2$ in solution of dialysed hæmoglobin and NaHCO$_3$ at varying CO$_2$ pressures.

	100 c.c. solution contain		
CO$_2$ mm.	Total CO$_2$ c.c.	Dissolved CO$_2$ c.c.	Combined CO$_2$ c.c.
4·8	52·6	0·3	52·3
4·9	52·4	0·3	52·1
11·0	58·3	0·8	57·5
21·9	64·8	1·5	63·3
40·7	75·7	2·8	72·9
43·6	79·5	3·0	76·5
50·7	79·0	3·5	75·5
52·5	73·9	3·7	70·2
78·1	79·5	5·4	74·1
85·5	85·8	6·0	79·8
90·0	83·4	6·3	77·1
110·5	100·0	7·7	92·3
119·8	94·6	8·3	86·3
151·4	104·0	10·5	93·5
221·9	114·5	15·4	99·1
226·7	113·6	15·8	97·8
310·4	123·5	21·6	101·9
338·0	130·5	23·5	107·0
435·3	140·3	30·3	110·0
596·4	153·7	41·5	112·2

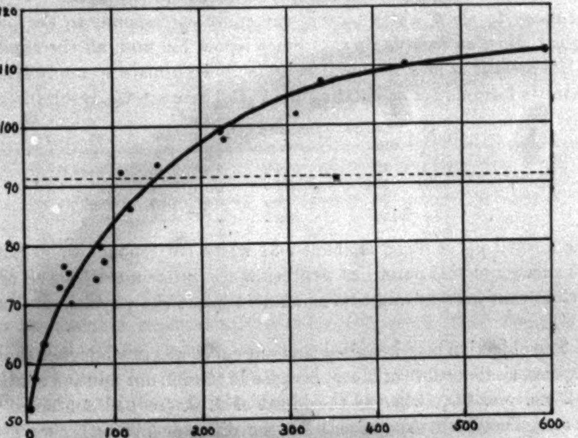

Fig. 1. Ordinate, percentage vol. CO$_2$. Abscissa, CO$_2$ press. mm.
• NaHCO$_3$ + dialysed Hæmoglobin.
Interrupted curve, NaHCO$_3$ alone.

The effect of adding dialysed red blood corpuscles to sodium bicarbonate is to diminish the amount of combined CO_2 below 150 mm. Thus at 4·85 mm. two independent results give a mean value of 52·2 vols. p.c. of CO_2, a reduction of 41·1 c.c. from the CO_2 in the pure sodium bicarbonate solution. This effect gets less and less, until at 150 mm. the curve cuts the interrupted line. This is the isoelectric point of the proteins of the corpuscles, and at pressures below 150 mm. the corpuscle proteins act solely as acid and all the CO_2 must be combined as sodium bicarbonate. At pressures above 150 mm. the solution takes up more CO_2 than the pure sodium bicarbonate. Hence the extra CO_2 must be combined directly with the protein. At 596·4 mm. for instance the combined CO_2 is 112·2 c.c. p.c. Now of this 91·3 c.c. is combined as sodium bicarbonate and the difference 20·9 c.c. is combined with the protein. As already stated we have been dealing with all the protein of the corpuscles mixed together, and although the hæmoglobin probably has the greatest effect, we have no proof of this. However from the physiological point of view the experiments are satisfactory because we have used the mixture of proteins which occurs naturally in the corpuscles. It is to be noted that the curve is even throughout its course. There is no sudden alteration in curvature at the isoelectric point.

Before these results can be applied to the blood in the body, it is necessary to show which part of the curve corresponds to the physiological range as regards its C_H. Since below 150 mm. all the combined CO_2 is present as bicarbonate it is perfectly legitimate to apply Hasselbalch's formula for calculating the C_H. These are the results:

CO_2 pressure mm.	Combined CO_2 c.c. p.c.	p_H
40	70·2	7·46
50	72·5	7·37
70	78·0	7·25
150	91·3	6·98

The normal p_H of blood is about 7·37 which corresponds to 50 mm. on the curve and this point lies well below the interrupted line, so that it is clear that hæmoglobin acts as an acid inside the physiological range of C_H.

The objection may be raised that our curve was much higher than the CO_2 dissociation curve of blood, because at 40 mm. our solution contained 70·2 c.c. p.c. CO_2, whereas the blood of J. J. (see (14)) contained only 45 c.c. There are two reasons for the difference. Firstly, we used a comparatively dilute hæmoglobin solution; if the concentration of this had been increased the CO_2 content would have been diminished.

Secondly, it will be shown later, that we were using too great a concentration of sodium bicarbonate. **Hasselbalch**'s formula shows that the p_H increases with the combined CO_2. If we had used less sodium bicarbonate the isoelectric point 6·98 would have been lower in Fig. 1, and with it all the points on the curve would have been shifted downwards. These objections do not of course invalidate our conclusions, which are based on the p_H of the solution and not on its CO_2 content. It is to be noted that **Michaelis** and **Takahashi**(18) found that the isoelectric point of hæmoglobin (separated from the protein of the stromá of the corpuscles) had a p_H of $-$ 6·745 which is decidedly more acid than the value we have obtained for all the proteins of the corpuscles together.

The combination of CO_2 *in serum.*

We have made some similar observations on dialysed serum from the defibrinated blood of healthy men. The serum was dialysed against distilled water and the volume was found to have increased from 77 c.c. to 128 c.c. There was a thick sediment of globulin, and there was less than ·02 gm. ash in 100 c.c. As with hæmoglobin we found that the solution of serum in distilled water combined directly with CO_2 but the amount of CO_2 combined was much less. In some of these experiments sodium chloride was first of all added to redissolve the globulin.

TABLE IV. Solution of dialysed serum and sodium bicarbonate.

	100 c.c. solution contain		
CO_2 mm.	Total CO_2 c.c.	Dissolved CO_2 c.c.	Combined CO_2 c.c.
11·8	85·3	0·7	84·6
27·7	86·1	1·9	84·3
47·0	88·7	3·3	85·4
114·0	99·1	7·9	91·3
188·0	102·3	13·0	89·3
290·5	112·9	20·2	92·7
436·0	119·6	30·2	89·4
706·0	145·6	49·1	96·5

The experiments with dialysed serum and sodium bicarbonate are given in Table IV. The sodium bicarbonate was the same strength as in the hæmoglobin experiments (91·3 c.c. CO_2 p.c.). This strength of $NaHCO_3$ caused the globulin to go into solution. The coefficient of solubility was calculated as 0·528 taking the dilution of the serum protein due to the dialysis into account. Three determinations with a pressure less than 50 mm. gave values for the combined CO_2, the highest of which was 85·4 c.c. p.c. This is 5·9 c.c. less than the CO_2 in the pure bicarbonate solution, so there is no doubt that the serum proteins act as acids at these pressures, though their effect is nothing like so striking as the hæmoglobin. At 290·5 mm. the combined CO_2 was 92·7 p.c. and at 706 it was 96·5 p.c., which means that at higher pressures the solution contains more CO_2 than the pure $NaHCO_3$ solution. Hence like hæmoglobin the serum proteins act as bases at these pressures. We obtained similar results using serum protein which had been precipitated, washed and dried by **Hardy** and **Gardiner**'s method (19), and redissolved as far as possible in distilled water.

Although it is quite clear from our results that serum proteins like hæmoglobin behave as amphoteric electrolytes, many more determinations would be necessary to settle the exact isoelectric point, especially as the differences of CO_2 involved are not so very much outside the experimental error of the method. We can only say that in our experiments it probably lies between 100 and 300 mm. CO_2. If 200 mm. is taken as the isoelectric point the calculated value for the p_H is 6·86. If 300 mm. is taken the p_H is 6·68. **Michaelis** (10) and his fellow workers again found by a different method much more acid values than this for the various serum proteins.

Discussion of results.

There would seem to be no doubt that both hæmoglobin and the serum proteins act solely as acids for a considerable distance on each side of the normal range of hydrogen ion concentration. Further the experiments with the hæmoglobin and bicarbonate solutions give data for calculating approximately the acid dissociation constant of oxyhæmoglobin. The reaction is represented thus:

$$Hb.H \rightleftarrows Hb + H$$

from which $\frac{(Hb) \times (H)}{(HbH)} = k$, k being the dissociation constant, the brackets indicating as usual the gramme molecular concentration of the various substances.

In the actual experiment a mixture of the free acid (oxyhæmoglobin) and its sodium salt is present. Under these circumstances (Hb) is the same as a (NaHb) where a is the degree of dissociation of the salt and (HbH) represents the concentration of free hæmoglobin, since the dissociation of the acid is now extremely small.

$$\frac{k}{a} = (H) \times \frac{(\text{sodium salt})}{(\text{free acid})}.$$

Sodium salts of acids are nearly completely dissociated, but although this dissociation will be rather hindered by the presence of excess of

Na in the form of bicarbonate, still a may be taken as unity as an approximation. The equation may be written thus:

$$p_K = p_H + \log \frac{\text{(sodium salt)}}{\text{(free acid)}} \quad \text{.................................(I)},$$

p_K being the exponential of the dissociation constant.

For calculating p_K a particular point on the curve in Fig. 1 is taken. For instance at 20 mm. the solution holds 63 c.c. p.c. CO_2. This CO_2 is present as bicarbonate. Now the total sodium in the solution combines with 91·3 c.c. CO_2 p.c. Hence at 20 mm. 91·3 – 63 = 28·3 c.c. CO_2 is equivalent to the amount of sodium combined with the hæmoglobin.

The free acid will be represented by the difference between the total amount of sodium that the hæmoglobin can combine with when there is no free acid left, and the amount of sodium salt actually present at 20 mm., *i.e.* 28·3 c.c.

The total combining power of Hb for Na is obtained by a slight extra-polation of the curve downwards to 40 c.c. This point has been taken for a reason that will be explained later. In other words it is assumed that when by reduction of the CO_2 pressure the solution only holds 40 c.c. CO_2 combined as carbonate or bicarbonate, the hæmoglobin is completely saturated with sodium. Hence the total hæmoglobin combining power will be represented by 91·3 – 40 = 51·3 c.c. The free acid will be 51·3 – 28·3 = 23·0 c.c. Now the p_H of the solution at 20 mm. calculated from Hasselbalch's formula is – 7·717. The equation (I) may be written:

$$p_K = -7{\cdot}717 + \log \frac{28{\cdot}3}{23} = -7{\cdot}627.$$

This same calculation may be carried out at other points on the curve. Two more have been taken, viz. at 50 mm. when the combined CO_2 is 73 c.c. p.c., and 100 mm. when it is 83·5 c.c. In the first case p_K is found to be – 7·631 and in the second – 7·873. Of course all these three values should really be the same and there is certainly an extremely close agreement between the first two. The main uncertainty in the calculation is the amount of extra-polation that should be carried out. The actual point 40 was taken because by using this value more concordant results were obtained for p_K at different parts of the curve than by using any other value. The uncertainty is increased by the fact that below 2 mm. the CO_2 is present at any rate partly as carbonate, and so it is not correct to say that 91·3 – 40 quite represents Na combined with hæmoglobin as some of this Na will have combined with the bicarbonate to produce normal carbonate. Some results for p_K may be given assuming that 50 c.c. (and not 40 c.c.) represents the point where all the hæmoglobin combining capacity for sodium has been satisfied. At 20 mm. $p_{\mathrm{K}} = -7{\cdot}38$ and at 55 mm. $p_{\mathrm{K}} = -7{\cdot}47$ and at 100 mm. $p_{\mathrm{K}} = -7{\cdot}76$.

It cannot be pretended that these calculations give more than a very rough idea of what the true dissociation constant is, but it probably is somewhere about $10^{-7\cdot7}$ or 2×10^{-8}. Unfortunately the data are not sufficient to calculate even approximately a value for the acid dissociation constant of the serum proteins.

Joffe and **Poulton** have given figures for the CO_2 content of J. J.'s "true serum" and red blood corpuscles. They have also pointed out that since the hydrogen ion concentration of blood as ordinarily measured is that of the true serum it can be calculated by means of **Hasselbalch's** formula. The present paper by showing that the serum proteins act as acids within the physiological range justifies this procedure. Now that hæmoglobin has also been shown to act solely as an acid it is also quite justifiable to use the formula for calculating the C_{H} of the red corpuscles.

TABLE V. The C_{H} of serum and corpuscles in J. J.'s oxygenated and reduced defibrinated blood.

	O_2 serum		O_2 corpuscles		Difference	Red serum		Red corpuscles		Difference	Serum difference	Corpuscle difference
CO_2 mm.	p_{H}	C_{H} $10^{-8}\times$	p_{H}	C_{H} $10^{-8}\times$	C_{H} $10^{-8}\times$	p_{H}	C_{H} $10^{-8}\times$	p_{H}	C_{H} $10^{-8}\times$	C_{H} $10^{-8}\times$	C_{H} $10^{-8}\times$	C_{H} $10^{-8}\times$
10	7·71	1·95	7·52	3·0	1·05	7·74	1·8	7·67	2·1	0·3	0·15	0·9
20	7·51	3·1	7·41	3·9	0·8	7·55	2·8	7·50	3·2	0·4	0·3	0·7
30	7·40	4·0	7·31	4·9	0·9	7·43	3·7	7·40	4·0	0·3	0·3	0·9
40	7·32	4·8	7·26	5·5	0·7	7·35	4·5	7·32	4·8	0·3	0·3	0·7
55	7·23	5·9	7·18	6·6	0·7	7·26	5·5	7·24	5·8	0·3	0·4	0·8
70	7·16	6·9	7·12	7·6	0·7	7·19	6·5	7·18	6·6	0·1	0·4	1·0
90	7·08	8·3	7·05	8·9	0·6	7·12	7·6	7·13	7·4	−0·2	0·7	1·5

Table V gives the results of these calculations both for oxygenated and reduced blood. Values both for p_{H} and C_{H} are given. It will be seen that at all pressures up to 90 mm. the corpuscles are slightly more acid than the serum but the differences are greater in oxygenated than in reduced blood, since oxyhæmoglobin is evidently a stronger acid than reduced hæmoglobin. At 40 mm. for oxygenated blood the p_{H} of the true serum is 7·32 and for corpuscles 7·26. At 40 mm. for reduced blood the p_{H} of true serum is 7·35 and for corpuscles 7·32. The difference in C_{H} between serum and corpuscles becomes less as the CO_2 pressure increases (cols. 6 and 11). In cols. 12 and 13 the difference between the oxygenated and reduced true serums are arranged and also between the oxygenated and reduced corpuscles. There is a bigger difference between the corpuscles than between the serums. It is true that we have only proved that oxyhæmoglobin acts as an acid but there can be little doubt that reduced hæmoglobin acts similarly, so that we have felt it justifiable to calculate the C_H of the reduced corpuscles as well as of the oxygenated corpuscles.

In Milroy's experiments the red blood corpuscles were found to be extremely acid. Thus at 49 mm. CO_2 the p_H was 6·60 or about 0·7 less than the p_H of the serum. It is hardly conceivable that there should exist such a big difference as this in the circulating blood. Milroy's procedure was responsible at any rate for a large part of the difference. He centrifuged off the corpuscles, and then to the corpuscular deposit he added enough water to bring back the volume to that of the original blood. Blood contains approximately 50 p.c. of its volume as corpuscles, so that he diluted the corpuscular volume to about double its original volume. This would halve the concentration of sodium bicarbonate in the corpuscles which from Hasselbalch's formula would reduce the true p_H by 0·3. This at any rate accounts for part of the great increase of acidity that he found.

Our experiments with solutions of sodium bicarbonate added to hæmoglobin and serum proteins provide a useful schema of the condition of affairs in the blood. At low pressures of CO_2 the hydrogen ion concentration of the hæmoglobin solution is greater than that of the serum proteins. For example at 20 mm. CO_2 the p_H of the hæmoglobin bicarbonate solution is 7·72. At this pressure the serum bicarbonate solution which contained 84 vols. p.c. CO_2 has a calculated p_H of 7·83. As the CO_2 pressure increases the CO_2 content of the hæmoglobin bicarbonate solution increases much more rapidly than the serum bicarbonate solution, so that the difference between the p_H's becomes less and less. Exactly the same thing is shown in Table V. At 20 mm. CO_2, the difference between the p_H's of serum and corpuscles of oxygenated blood is 1·0, at 90 mm. it is only 0·03.

As has already been pointed out (14) the condition in reality is much more complicated in blood, because there is an interchange of ions constantly occurring between corpuscles and plasma. The reason for this interchange taking place is clear, though the mechanism of its production is not clear. In the corpuscles the hæmoglobin is nearly four times as concentrated as in our solution and so the dissociation curve must be steeper. This means that raising the CO_2 pressure causes a greater increase in the CO_2 content of the corpuscles, so that the hydrogen ion concentration is more rapidly diminished. This excessive alteration in C_H is prevented by diffusion of acid from the serum.

It is clear from our experiments why all the CO_2 can be expelled from blood by means of the blood pump. The hæmoglobin of the corpuscles is present in sufficient quantity to combine with all the available sodium of the blood, as the CO_2 is gradually removed. In our experiment the concentration of hæmoglobin was less than in blood and the amount of available sodium was greater, so that there were still 40 c.c. CO_2 combined with sodium when the CO_2 pressure was reduced to zero. In blood the interchange of ions between plasma and corpuscles ensures that on reduction of pressure the residue of available sodium after the plasma proteins have been satisfied, receives acid from the corpuscles and so makes sodium available inside the corpuscles to combine with the hæmoglobin. In the separated serum the proteins are not sufficient to combine with all the available sodium, so that on reduction of pressure some CO_2 is still left in combination with it.

The experiments with hæmoglobin and serum proteins also give an indication of the total amount of sodium bicarbonate in blood, or, expressing it more accurately, the total amount of sodium available for combining with CO_2. Parsons(8) has suggested that the sodium bicarbonate is $0{\cdot}045N$ which corresponds to the total combined CO_2 when blood is completely saturated with CO_2. This suggestion takes no account of the fact that while all the sodium of the blood will be present as sodium bicarbonate additional CO_2 will be taken up in combination with the hæmoglobin and serum proteins when the hydrogen ion concentration is increased to this extent. On the other hand at the isoelectric point all the sodium is present as bicarbonate, and no CO_2 is combined with protein.

It was pointed out that our experiments with the serum-bicarbonate solution showed that the most probable value for the p_H of the isoelectric point was 6·86. Now calculations show that this value corresponds to 180 mm. on the true serum curve of J. J.'s blood (cf. (14) Fig. 3). At this pressure the difference between curves B and C which gives the CO_2 content in the serum of 100 c.c. blood, is 37·9 c.c. and this corresponds to the total available sodium in the serum, since this is the isoelectric point of the serum proteins. The CO_2 in the corpuscles at this pressure will be partly present as bicarbonate and partly combined with hæmoglobin, since the reaction of the corpuscles at this pressure is more acid than the isoelectric point of the corpuscles, viz. 6·98.

Now the amount of CO_2 in the corpuscles combined as bicarbonate will be the volume of CO_2 in the corpuscles at the isoelectric point. Calculation by means of Hasselbalch's formula shows that when the

p_H of the corpuscles is 6·98 the pressure is 117 mm. corresponding to 53·6 c.c. combined CO_2 in 100 c.c. corpuscles. The corpuscles in 100 c.c. blood at 117 mm. contain 29·9 c.c. CO_2 (cp. (14), curve C, Fig. 3), this represents the total available sodium in the corpuscles of 100 c.c. blood. The total available sodium in 100 c.c. of blood is the same as that in the serum and corpuscles, and is equivalent to $37·9 + 29·9 = 67·8$ c.c. CO_2, which means that the total sodium bicarbonate of blood is 0·03*N*.

There is admittedly some uncertainty in the calculation because our experiments with the serum-bicarbonate solution were not sufficient to settle accurately the isoelectric point of the serum proteins, and no allowance has been made for any diffusion of acid from corpuscles to plasma between the two isoelectric points. However we think that the value we have obtained (0·03*N*) is probably more nearly correct than that calculated by Parsons, viz. 0·045*N*, which is almost certainly a good deal too high.

CONCLUSIONS.

1. The proteins of blood combine directly with CO_2 when the reaction of the blood is very much more acid than ever occurs in the body.

2. Throughout the range of C_H occurring in the body all the CO_2 is present as bicarbonate, and the blood proteins act as acids combining with sodium and competing for it with the CO_2.

3. Experiments with dialysed red cells and sodium bicarbonate indicate that the isoelectric point of hæmoglobin has the value $p_H = 6·98$. The acid dissociation constant of hæmoglobin is 2×10^{-8}.

4. At all pressures of CO_2 the C_H of the corpuscles is greater than the C_H of the plasma. The difference becomes less as the pressure rises.

5. CO_2 is completely expelled from blood by the blood pump, because the blood proteins in their capacity as acids are present in sufficient concentration to combine with all the sodium of the blood.

6. The maximum possible quantity of sodium bicarbonate in blood (*i.e.* the total sodium available for combining with CO_2) is 0·03*N*.

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COLORIMETRIC DETERMINATION OF THE REACTION OF BLOOD BY DIALYSIS. BY H. H. DALE AND C. LOVATT EVANS.

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DURING recent years measurement of the reaction of blood has mostly been carried out with the hydrogen electrode. Whether a small bubble of hydrogen is used, brought into equilibrium with the blood in respect of CO_2 by shaking (Hasselbalch(1), Michaelis and Davidoff(2)), or whether an artificial mixture is made of hydrogen and CO_2 at the tension of alveolar air (Peters(3)), and the blood brought into equilibrium with this, the film of blood in which the reaction is actually measured is in the reduced condition. When the latter method is used, the correspondence of the reaction so determined with that of the circulating blood is evidently conditioned by the accuracy with which the CO_2 tension in the lung alveoli can be determined. This is true also of indirect methods such as that of Barcroft(4), in which the hydrogen ion concentration of the blood exposed to an artificial gas mixture is determined from the constant, K, of the oxygen dissociation curve.

The idea of avoiding the difficulty, occasioned in the use of indicators by the colour of blood or plasma, by testing a colourless dialysate in place of the blood itself, appears to be quite an old one. It was used by Kühne(5) as long ago as 1865. To Levy, Rowntree and Marriott(6), however, belongs the credit of having introduced its use with modern indicators, capable of giving a direct measure of the hydrogen ion concentration of a fluid, by matching with a series of standard solutions containing the indicator. The method, as devised and used by them, suffered from the lack of any serious precaution to avoid loss of CO_2 either from the blood or serum placed in the dialyser, or from the dialysate of which the reaction was determined. For our purpose, which was to study the variations, from minute to minute, of the reaction of the circulating arterial blood, in an animal exposed to different conditions, determinations thus taken would clearly have little, if any, value. R. W. Scott(7), who had a similar object to ours, modified the dialysis method so as to restrict very greatly the error due to loss of CO_2. It seemed to us that relatively small modifications ought to make the method one of real precision.

In this paper we give the working details of the modifications which we have found desirable and effective to this end, and of some results by which we have tested the performance of the method, and from which an idea of its accuracy can be obtained. Concerning the convenience of the method as we have used it there can be no doubt: we believe that, even in accuracy, it is likely to prove little if at all inferior to the more elaborate and less direct methods which others have used.

The essential apparatus consists of a flat-bottomed cylindrical vessel, which we call a comparator vessel, into which the tubular dialysing membrane fits loosely. The membrane is tied on to a vulcanite holder, which closes the top of the vessel, and is itself closed by a cork.

The comparator vessels. These were made from glass tubing with an external diameter of 14 mm. and internal diameter of 11·5 mm. Pieces of equal length were cut from this, the ends ground flat, and to one end of each a circular disc cut from a white microscope slide was cemented with Canada balsam. It is necessary, when the balsam has hardened, to remove any excess from the interior of the vessel, as the balsam is apt to absorb indicator and vitiate the colour comparison when the tube is in use. Better tubes could no doubt be made with flat bottoms of optically worked glass, but we have not found it possible to get them under present conditions. The length of the finished vessels, as we have used them, is 40 mm.

The vulcanite membrane holder. This is turned from a single piece of vulcanite. The shape can be seen from Fig. 1 A (full size), from which measurements can be made. It will be seen that there is a central tube of vulcanite, the upper orifice of which can be tightly closed by a small cork. The tube has a groove cut round it some 2 mm. from the lower end, to facilitate the tying on of the membrane. The upper end has a wide thick flange, the under surface of which is cut into a deep annular groove, in such a way that the upper end of the comparator vessel passes into the groove, of which the outer wall fits fairly closely over the glass vessel, while sufficient space is left between the latter and the central vulcanite tube to accommodate easily the membrane and the ligature securing it.

The dialysing membrane. This is made of collodion on the inside of a small test-tube, which is carefully selected by measurement of such a size that the finished dialysing membrane will be very slightly smaller in diameter than the comparator vessel, the space between the two being such that 1 c.c. of fluid will fill the annular space around the dialyser without actually reaching to the top of the vessel. The tube which we have employed for making the membranes has a length of about 60 mm., and an internal diameter of 11 mm. The membrane is made as follows: the tube is filled almost to the top with a strong solution (about 10 p.c.) of collodion in alcohol-ether, air bubbles are removed by a brief centrifugation, and the film which has formed on the surface of the solution is peeled off with forceps; the tube is then inverted and allowed to drain out of contact with the air. If the tube is inverted in the mouth of the bottle containing the solution, drainage takes place in the vapour of the solvent, and a thin membrane is obtained. When the collodion film is sufficiently inspissated (if desired drying may be hastened by a gentle stream of air, after drainage is sufficiently complete), the tube is filled with water. If a specially permeable membrane is desired, the tube, after draining in the vapour of the solvent, is filled

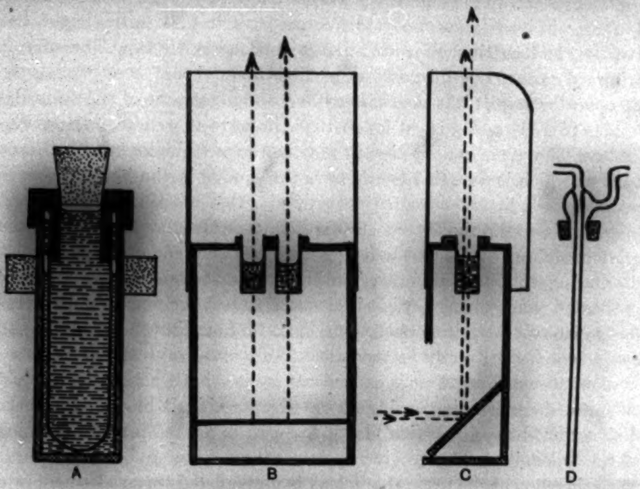

Fig. 1 A. Section of dialyser and comparator vessel filled for a determination. (Full size.)
B, C. The comparator. (One-fourth full size.)
D. Attachment for dialysis *in vivo*. (Full size.)

with 70 p.c. alcohol and allowed to stand (cf. Brown(8)). The membrane is in either case thoroughly washed in water, detached from the rim of the tube, separated from the rest of the tube by allowing water to pass between the membrane and the glass, removed and kept in water until required. The membrane is cut to the required length to fit the comparator vessel and is then tied on to the vulcanite cap, which closes the top of this vessel and prevents the escape of carbon dioxide.

The membrane is cut to the proper length by filling it with water, lowering it into the comparator vessel until it just rests on the flat bottom, and cutting off with scissors level with the top. The edge is trimmed so that the finished membrane is about 1 mm. shorter than the comparator vessel; it is then slipped over the inner tube of the vulcanite holder (Fig. 1) and pushed up so that the edge is well past the tying-groove. The membrane is tied in position by a stout linen thread, one turn of which is first tied firmly over the groove in the holder so as to press the collodion membrane tightly into it. One end of the thread is left long, and is wound tightly in a close spiral round the membrane above the groove, and finally secured by tying it to the other end of the first knot.

During this and all other manipulations the membrane must be prevented from getting dry. It is essential to test the finished membrane for leakages, due to imperfect tying or to pinholes in the collodion, and also to confirm its ready permeability to dissolved crystalloids.

Test of soundness. This is carried out by making a blank dialysis just as for a determination, using oxalated or whipped blood. After the dialyser has been filled with blood, the cork is pushed into the opening of the vulcanite holder to give an internal pressure, and so to increase the stringency of the test. If any leak is present, red corpuscles pass into the saline and are easily detected.

Test for permeability. A dummy mixture, either of phosphates of P_H. 7·5–8·0, or else a 0·25 p.c. sodium bicarbonate solution exposed to alveolar air, is run into the dialyser and dialysed against the saline solution for five minutes. At the end of this time the dialysate should give the same colour as the original fluid when neutral red is added in equal amounts to both, and a comparison made in the comparator.

The comparator (Fig. 1 B, C). This consists of a small box with blackened interior, the top of which is perforated by two holes placed close together to receive the comparator vessels. The lower portion of the front wall of the box is open to admit light, which is reflected up through the tubes by a sheet of opal glass placed at an angle of 45°. Around the top of the box is a blackened screen to exclude light from above.

Method of use. 1 c.c. of a ·85 p.c. solution of NaCl in freshly boiled distilled water is placed in a comparator vessel. The membrane on its holder, which has meanwhile been thoroughly soaked in the saline solution, is quickly shaken free from adherent fluid, both inside and outside. It is then corked to prevent collapse, and lowered into the comparator vessel, the holder being pressed down till the upper rim of the vessel reaches the bottom of the groove in the holder; should the groove fit too loosely over the vessel, plasticine may be put into it to make an airtight junction. The cork is now removed and a pinch of powdered carbonate-free potassium oxalate is dropped into the bottom of the dialyser. The stock of oxalate should be tested for neutrality by the addition of neutral red to a 0·1 p.c. solution of the salt. The colour given should not differ appreciably from that given by water alone.

The blood is withdrawn from the vessel by a long, slightly bent and narrow cannula, which may with advantage also contain a few milligrammes of oxalate. This cannula is inserted into the clamped artery or vein and tied in. The clamp is carefully released and blood allowed to fill the cannula without the inclusion of air-bubbles. The open end of the cannula is then passed to the bottom of the dialysing membrane and the clamp controlled so that the membrane is steadily filled from below upwards with blood until it reaches almost to the top of the opening in the vulcanite holder, the point of the cannula being always kept below the surface of the fluid. The opening is then immediately closed, either with a small cork or with a stopper provided with a narrow glass capillary (Fig. 1 A). Dialysis is now allowed to proceed for ten to fifteen minutes.

Treatment of the dialysate. When dialysis is complete the dialysing membrane with its contents is removed and put into a vessel of 0·85 p.c. NaCl, where it is left until it can be washed. To the dialysate a few drops of neutral red solution are added from an accurate dropping pipette. Individual tastes will probably differ as to the amount of neutral red to be added, but we have recently used four drops (=0·08 c.c.) of a 0·02 p.c. solution in distilled water. Some workers may very probably prefer to use phenol red (phenolsulphonephthalein) for this comparison. We found that neutral red gave us a more finely graded scale of colour-differences within the range of reactions which concerned us. The drops are added quickly, and after proper admixture the fluid is immediately covered with a layer of pure liquid paraffin; this effectively prevents any loss of carbon dioxide sufficient to change perceptibly the tint of the neutral red in the dialysate, for a period far beyond that needed. It has often been pointed out that carbon dioxide is by no means insoluble in liquid paraffin, but this fact does not affect its use for this purpose. The liquid paraffin prevents escape of CO_2 only by greatly restricting diffusion from the surface, passage of CO_2 from the water to the oil being rendered very slow by the relatively high viscosity of the latter.

The comparative titration. The determination of the P_H of the dialysate, is carried out by comparison with the tint of a phosphate mixture containing an equal amount of neutral red, and placed in another similar comparator vessel. The reaction of this phosphate mixture is adjusted by titration until an exact colour match is obtained. There are many ways in which such titration could theoretically be carried out. For example, the original Sörensen (9) mixtures could be used, starting with either the disodium or the monopotassium phosphate and adding the other solution until the correct colour was reached. We found, however, that this would in some cases necessitate the use of inconvenient volumes or proportions of the two fluids, and, further, that the sharpness of the end-point would vary inconveniently at different ranges. We have found it convenient therefore to employ three phosphate mixtures with P_H values, as follows: (1) P_H 7·5, (2) P_H 6·5, (3) P_H 10·5. Solutions Nos. (1) and (2) can be prepared, if desired, by mixing the two Sörensen $\frac{1}{15}$ molar phosphate solutions in the calculated proportions, but we have found it more convenient (and essential in the preparation of No. 3 solution) to start with acid potassium phosphate, which is readily obtained pure, and to add standard sodium hydroxide solution (free from carbonate) in the required proportion, according to the suggestion of Clark and Lubs (10).

The following are the proportions required to obtain solutions $\frac{1}{15}$ molar phosphate-ion, calculated from Sörensen's curves in the case of solutions (1) and (2), and from data given by Prideaux (11) in the case of solution (3), which for obvious reasons cannot be obtained from Sörensen's mixtures. We are greatly indebted to Dr C. J. Martin, F.R.S., and to Dr C. G. L. Wolf, who kindly assisted by checking some of our solutions with the hydrogen electrode.

Solution No.	P_H.	c.c. M/5 KH_2PO_4	c.c. normal NaOH	c.c. N/10 NaOH	c.c. freshly boiled distilled water
1	7·5	33·33	—	56	to 100
2	6·5	33·33	—	21·33	to 100
3	10·5	33·33	7·33	—	to 100

In order to prevent the growth of moulds, a trace of calomel may be added to each bottle of the phosphate solutions as suggested by Martin.

The vessel containing the dialysate is placed in one of the holes of the comparator box, and in the other hole is a similar tube containing 1 c.c. of the No. 1 phosphate solution (P_H 7·5) to which the same number of drops of neutral red have been added. The contents of this vessel are thoroughly mixed with a thin rod of hard glass, or better with a small platinum stirrer[1], and it will be at once obvious whether the dialysate is more or less alkaline than P_H. 7·5. The reaction of the No. 1 solution is now altered by adding drop by drop from a finely pointed pipette graduated in $\frac{1}{100}$ths of a c.c., with constant stirring, either solution No. 3 or solution No. 2, until the colours in the two tubes exactly match. The quantity of solution No. 3 or No. 2 added is then read off on the pipette, and the P_H of the mixture so prepared to match the dialysate is ascertained from one of the accompanying graphs (Figs. 2 and 3).

It is important to note that these graphs are intended for use with solutions made up as indicated, to be $\frac{1}{15}$th molar. The curve given by Prideaux (11), and reproduced in extended form by Bayliss (12), gives, for the same proportions of alkali to phosphoric acid in $\frac{1}{10}$th molar solutions, figures for P_H. which differ materially from those of Sörensen. The reason for this difference is still somewhat obscure: the difference in concentration seems inadequate to account for it. It is, however, for the present advisable to use the determinations of any author only for solutions containing the same ions in the same molar concentration as he indicated.

The whole procedure is very rapid, and duplicate results obtained by both of us, working independently, seldom show a wider discrepancy than P_H 0·03, while they usually correspond to within P_H 0·02.

By a slight modification of the apparatus (Fig. 1 D) the method can be made available for determining the reaction of arterial blood of the anæsthetised animal during actual circulation. For this purpose the membrane holder is provided with the small attachment shown in the figure, which enables the arterial blood to be led down to the bottom of the dialyser tube, out through the side tube at the upper end, and so back *viâ* a bubble-trap into the circulation. We believe this arrangement may be found useful for certain physiological enquiries, and the only circumstance which has hindered us from applying it ourselves in a series of experiments is the present lack of efficient hirudin.

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[1] A convenient stirrer is made by welding a piece of platinum foil 5 mm. square on to a piece of stout platinum wire 20 or 30 mm. in length. This can be accomplished by placing the wire and foil in apposition, supported on an anvil, heating to bright redness in the blow-pipe flame, and striking one smart blow with a hammer. The wire is then mounted on a convenient handle of glass or metal.

Another application which we hope the method will find is the determination of the reaction of human arterial blood under different conditions. Now that the removal of arterial blood into a syringe by arterial puncture is so often performed, it should be a very simple matter to run the blood from the syringe into the dialyser with as little contact with the air as that entailed by bleeding from an arterial cannula. When once such a sample has been obtained and set to dialyse, 15 to 20 minutes should suffice for the completion of the determination.

Examples of results. Pending the publication of the results of the experiments for which we elaborated the method, it may be of interest

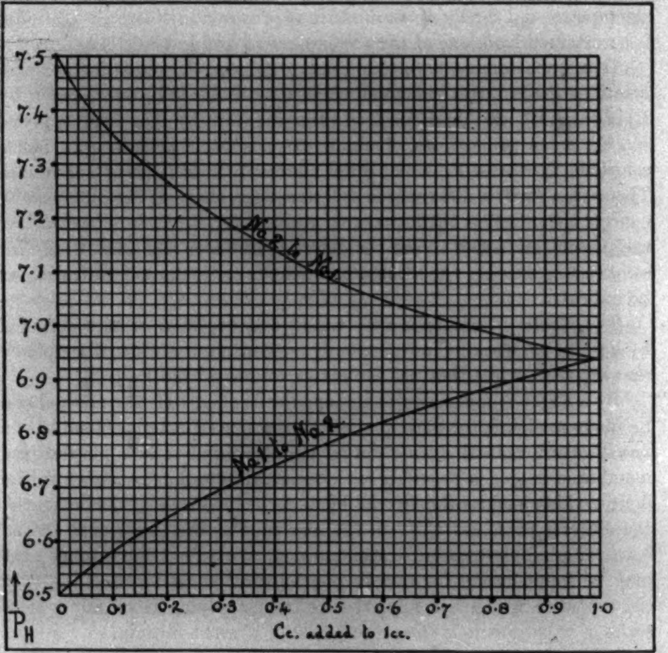

Fig. 2. Curve showing $P_{H^\cdot}$ of mixtures of Solutions 1 and 2. The curve is calculated from the data given by Sörensen for mixtures of disodium and monopotassium phosphates in $\frac{1}{15}$th molar solutions. Upper curve—addition of Solution 2 to 1 c.c. of Solution 1. Lower curve—addition of Solution 1 to 1 c.c. of Solution 2.

to give some results which will convey an idea of the absolute values obtained for blood and other solutions by its use. Fig. 4 shows a curve for the P_{H}. of cat's blood at different tensions of carbon dioxide. The determinations were made by rotating the blood in a Barcroft tonometer in which the desired mixture of air and CO_2 was made up in the usual way. The rotation was carried out at room temperature, and, at the end of 30 minutes or more, a sample of the gas was withdrawn for analysis, and a part of the blood used for the determination of the reaction by the above method. It will be seen that the points fall fairly well into alignment on the curve. In order to obtain some results which might be checked, either by theoretical methods, or by comparison

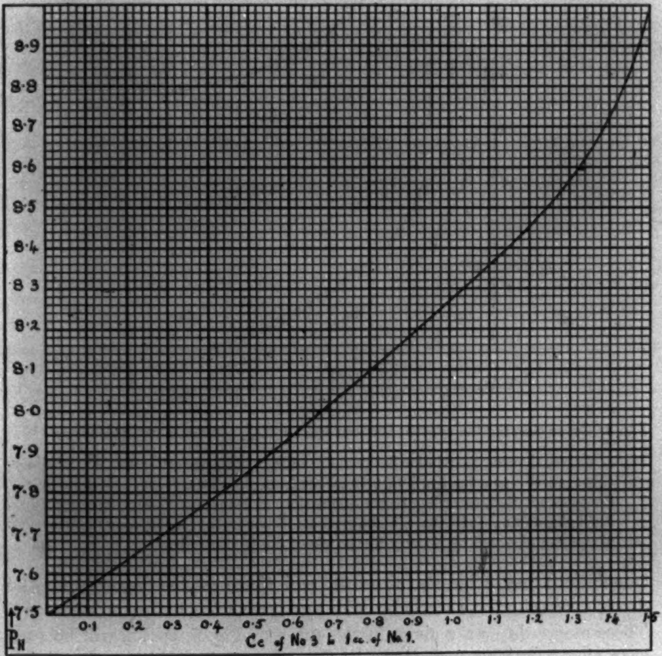

Fig. 3. Curve showing the P_{H}· of mixtures of varying amounts of Solution 3 with 1 c.c. of Solution 1. Calculated from Sörensen's data.

with determinations by the hydrogen electrode, we have investigated the P_H.-CO_2 curves of (1) 0·02 m. $NaHCO_3$ in distilled water and (2) 0·02 m. $NaHCO_3$ solution in 0·18 m. NaCl solution. The results of the former determination could then be compared with the theoretical

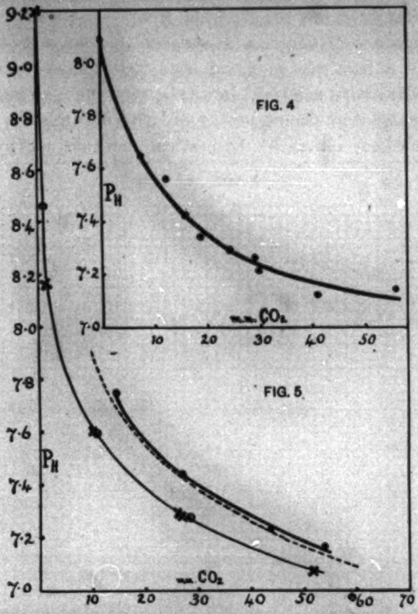

Fig. 4. Curve showing the relation between P_H· and CO_2 tension in cat's blood.

Fig. 5. Curves showing the relation between P_H· and CO_2 tensions for bicarbonate solutions. Upper curve = aqueous $NaHCO_3$ 0·02 molar, from our observations. Dotted curve = the same solution, according to the calculations of Parsons. Lower curve = 0·18 m. NaCl plus 0·02 m. $NaHCO_3$; × = observed points (corrected for salt error of indicator); ⊙ = points observed by Milroy by the use of the H electrode.

calculations made by Parsons(13), and those of the latter with the direct determinations made by Milroy(14) with the hydrogen electrode. These comparisons are given in the curve of Fig. 5, and it will be seen that the agreement, especially in the case of the solution containing sodium chloride, is very good.

We are indebted to our colleague, Dr John Brownlee, for the services of his department in the preparation of the graphs for Figs. 2 and 3.

Some of the apparatus used in this work was purchased out of a grant from the Government Grants Committee of the Royal Society to one of us (E.).

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THE CARBON DIOXIDE CARRYING POWER OF THE CONSTITUENTS OF PLASMA. THE ALKALI RESERVE OF BLOOD.

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THE hypotheses put forward to elucidate the relations of carbon dioxide in blood may be briefly summarised into three categories: (*a*) that carbon dioxide is associated with the diffusible and non-diffusible alkali of blood (Loewy and Zuntz(1)); (*b*) that a considerable portion of the carbon dioxide is in combination with hæmoglobin (Bohr(2), Buckmaster(3)); and (*c*) that carbon dioxide is present in blood entirely in the form of bicarbonate (Haldane, Hasselbalch(5), Parsons(6)). The last hypothesis has recently been analysed from a mathematical standpoint by Parsons(6) on the assumption that the laws of mass action may be applied to mixtures of colloid and crystalloid substances contained in a fluid so complex as blood. On this assumption he compared the carbon dioxide dissociation curve of a solution of sodium bicarbonate with that found by Christiansen, Douglas and Haldane(4) for blood. He concluded that the whole of the combined carbon dioxide of blood is present in the form of sodium bicarbonate and that the proteins of blood confer on this bicarbonate the property of being an efficient carrier of carbon dioxide. The fundamental basis of the bicarbonate hypothesis is that the proteins of blood act as weak acids, and by virtue of this property cause sodium bicarbonate to dissociate at tensions of carbon dioxide present in alveolar air. This hypothesis therefore is complementary to that put forward by Moore(7) that the maintainance of the neutrality of blood is one of the main functions of the protein of blood. But

Bayliss(8) observed that the proteins of plasma do not function as acids or alkalies at hydrogen ion concentrations compatible with life. Therefore the bicarbonate hypothesis, put forward to explain the carbon dioxide carrying power of blood, must be modified to the extent that the second weak acid which shares the sodium with the carbon dioxide must be associated with the corpuscular elements of the blood. In a later communication **Parsons**(9) states that the protein of blood which confers on bicarbonate the capacity of being an efficient carrier of carbon dioxide is mainly, if not entirely, hæmoglobin. This hypothesis, that hæmoglobin functions as an acid capable of decomposing sodium bicarbonate, is directly opposed to the conclusions of **Buckmaster**(3) that the transport of carbon dioxide is effected mainly by hæmoglobin.

Methods. The experimental results were obtained by determining the capacity of blood and its constituents to combine with carbon dioxide. The capacity of any fluid to carry carbon dioxide was found by exposing it to human alveolar air and then determining the total quantity of carbon dioxide contained in it by van Slyke's method. In the results submitted the amount of carbon dioxide in solution is not included since the object of the experiments was to determine the manner in which carbon dioxide is combined in blood. In the case of fluids carrying a small quantity of carbon dioxide in combination the amount dissolved forms a considerable portion of the whole and gives an erroneous impression of magnitude. In many cases the plasma fractions were ashed and the capacity of the ash, when dissolved in water, to combine with carbon dioxide was determined. In regard to these experiments it may be observed that the values so obtained give the maximum quantity of carbon dioxide which could be carried by the alkaline salts in the original fluid. It is clear that the organic salt of a metal of the alkali group, although incapable of carrying carbon dioxide in the original fluid, yet when ashed yields free alkali capable of combining with carbon dioxide.

The permeability of the red blood corpuscles.

The ash of serum. The bicarbonate hypothesis for the transport of carbon dioxide in the blood depends upon the assumption that a ready interchange of anions or kations takes place across the envelope of the red blood corpuscles. The fact that red blood corpuscles circulate in the vascular system in a fluid medium has led to the assumption that such an interchange of ions takes place across the cell envelope. In the case of the gases of blood, oxygen and carbon dioxide, the fact is self-evident, but with regard to inorganic anions and kations as Cl, P_2O_5, K.Na, etc., the proofs of free interchange are not readily obtained. **Hamburger**(10) stated that red blood corpuscles were permeable to Cl and P_2O_5, even in isotonic solution, and that when CO_2 was added to blood Cl passed from the serum to the corpuscles to preserve the neutrality of blood. **Höber**(11), on the other hand, stated that under normal conditions the red blood corpuscle is impermeable to anions, and permeability is produced only by treatment with CO_2. **Gürber** found that the red blood corpuscles were impermeable to K and Na, and his results were confirmed in 1904 by **Höber**(12). In 1909 **Hamburger** and **Hekma**(13) stated that small quantities of Ca may penetrate into red blood corpuscles, but **Abderhalden** and **Fränkel** state that Ca is found only in the plasma. Recently **de Boer**(14) has demonstrated the passage of small quantities of SO_4 from the plasma into the corpuscles. It may be concluded, therefore, from the results of experiments on the permeability of red blood cells to anions and kations that interchange cannot be demonstrated with ease and certainty. The fact that no such interchange readily occurs can be shown by a simple experiment. On the addition of red blood cells to sodium chloride solutions of varying strengths hæmolysis usually occurs at ·55 p.c. NaCl. The experiment may be repeated in another form, thus, a drop of blood is added to 10 c.c. of ·9 p.c. NaCl; after half an hour water is added to the mixture to bring the salt content to ·8 p.c. NaCl. A similar addition of water reducing the NaCl content ·1 p.c. is repeated every half hour. It is found that hæmolysis occurs at ·55 p.c. NaCl in this case also. If any interchange of anions or kations took place across the surface of the corpuscle it is clear that hæmolysis would occur with a lower concentration of sodium chloride in the second case—in fact, with perfect interchange the corpuscles could be obtained intact in pure water. In the section dealing with the ash of serum obtained from normal blood and from blood freed from CO_2 it is evident that under these conditions also no Na or Cl passes from the serum to the corpuscle or from the corpuscle to the serum. The strength of the envelope of the red blood corpuscle, shown by the low percentage of NaCl (·55 p.c.) required to produce hæmolysis, indicates that conditions may arise when the contents of the corpuscle are not in equilibrium with the plasma. Therefore the general consideration of the permeability of the red blood corpuscle to anions and kations does not lead to conclusions favourable to the bicarbonate hypothesis which postulates that hæmoglobin functions as an acid and thereby shares the available sodium of the blood with carbon dioxide.

(*a*) Normal serum. Experiments have been made to determine the relative capacities of serum and the inorganic salts contained in it to carry carbon dioxide. In order to obtain a solution of the inorganic salts a known volume of serum was dried and ashed at a dull red heat. The ash was dissolved in a volume of water equal to that of the original serum. The solution was exposed to alveolar air until equilibrium was attained and the amount of carbon dioxide contained in it was compared with that obtained from the original serum similarly treated. The following figures give the values obtained in two typical experiments:

Cat's blood	Serum (alveolated)	46·3 % CO_2
	Solution of ash (alveolated)	46·2 % „
Rabbit's blood	Serum (alveolated)	32·1 % „
	Solution of ash (alveolated)	32·4 % „

The identity of the values giving the capacity of serum and of the inorganic salts contained in it to carry carbon dioxide appears to offer strong evidence in favour of the hypothesis that all the carbon dioxide of blood exists in combination with sodium. The acceptance of this hypothesis from the above figures would, however, demand that no organic acid such as lactic acid should exist in the serum in combination with sodium. That such a condition should exist is improbable—in fact, later experiments show that serum always contains lactic acid.

(*b*) The ash of serum obtained from blood containing no carbon dioxide. Parsons' hypothesis that the sodium of blood is shared between the two weak acids hæmoglobin and carbon dioxide according to the law of mass action can be readily tested by experiments similar to those quoted for serum. On this hypothesis blood freed from carbon dioxide should have all its available alkali combined with the hæmoglobin. Since the corpuscles can be removed by centrifuging the serum from CO_2-free blood should contain much less inorganic salt capable of combining with carbon dioxide than serum obtained from blood containing a normal quantity of carbon dioxide.

	I	II
Serum from normal blood:		
Capacity of solution of ash to carry carbon dioxide ...	43·0 %	46·2 %
Serum from CO_2 free blood:		
Capacity of solution of ash to carry CO_2	42·0 %	44·6 %

It is evident from these figures that on depriving blood of carbon dioxide the sodium does not leave the serum to enter into combination with the hæmoglobin contained in the corpuscles. These results have been explained by assuming that when carbon dioxide is removed from blood a constant reaction is preserved, not by the entrance of sodium into the corpuscle, but by the exit of chlorine from them into the plasma. This explanation accords with the statement of **Hamburger**(10) that the red blood corpuscle is permeable to the Cl ion. This hypothesis is disproved by two facts—(1) the constancy of the available alkali contained in the ash of serum obtained from normal blood and from CO_2-free blood, and (2) the identity of the Cl content of serum obtained from normal blood and CO_2-free blood. (i) The figures above give the alkali contained in the ash of serum available for combining with CO_2 and not the total sodium. Whether the sodium ion passes into the corpuscle from the serum or the chlorine ion passes into the serum from the corpuscle, the alkali contained in the ash of serum available for combining with CO_2 would be diminished to the same degree. The available alkali contained in the ash of normal serum is identical with that in the ash of serum obtained from CO_2-free blood. (ii) Direct experiment shows that there is no difference in the chlorine content of serum of normal blood and serum obtained from CO_2-free blood. The relative quantities of chlorine present in the serum of normal blood and in the serum from CO_2-free blood were in the ratio of 100 to 95. The two figures are equal within the limits of experimental error. The comparative results obtained by analysing the ash of normal serum and that obtained from CO_2-free blood offer no evidence in favour of the hypothesis that hæmoglobin acts as a weak acid and shares with CO_2 the available free alkali in the blood. The results can be explained on the bicarbonate hypothesis only by assuming that the proteins of blood plasma, and not the hæmoglobin contained in the red blood corpuscle, function as the second weak acid. This alternative hypothesis is considered in the experiments dealing with the capacity of serum proteins, obtained from normal and CO_2 free serum, to combine with CO_2.

Lactic acid in blood. The presence of lactic acid in blood has been demonstrated by many observers. Probably the most accurate experiments on this subject are those of **Ryffel**(15), who found that resting human blood contained ·012 p.c. lactic acid whilst blood drawn after exercise contained ·07 p.c. lactic acid. The question was investigated to determine (i) the cause of the steady diminution in the CO_2 capacity of shed blood, (ii) the change which occurs in blood after the removal of CO_2, as evidenced by a diminished capacity to recombine with CO_2, and (iii) the effect of adding lactic acid to blood on its capacity to carry CO_2.

(i) Changes in the carbon dioxide capacity of drawn blood. **Christiansen, Douglas and Haldane**(4) have drawn attention in their paper (p. 246) to the fact that the capacity of blood to carry carbon dioxide diminishes after removal from the body. They offer no explanation of this fact. The degree to which shed blood loses its capacity to carry carbon dioxide is illustrated by the following experimental results:

Interval after removal from animal	CO_2 capacity
·5 hour	49·4 % CO_2
1·5 hours	46·8 % „
3·5 „	38·0 % „
5·5 „	32·0 % „

This steady fall in carbon dioxide capacity might be due to the production of lactic acid in blood comparable to that produced in dying tissues. The quantity of lactic acid in blood was therefore determined at different intervals of time. Considerable variations in the quantity of lactic acid in fresh blood have been observed. The following figures show typical results:

	A	*B*
Fresh blood	·07 % lactic acid	·1 % lactic acid
Blood after 3 hours ...	·18 % „ „	·133 % „ „

The blood used in the above experiments was obtained from anæsthetised cats. The lactic acid values for fresh blood in cats (*A*) and (*B*) presumably represent the different degrees of asphyxia induced by the anæsthetic in the two cases. In both cases, (*A*) and (*B*), the lactic acid present in the blood augmented on standing, indicating that blood is like every other tissue of the body in so far that lactic acid is produced in it on dying. It may be observed that ·1 p.c. lactic acid is capable of combining with sufficient sodium bicarbonate to diminish the CO_2 capacity of blood 24 p.c. Therefore the production of lactic acid in dying blood is capable of accounting for the steady diminution in the carbon dioxide capacity of blood after removal from the body.

(ii) The diminished capacity of serum obtained from CO_2-free blood to combine with CO_2. Serum obtained from CO_2-free blood combines with less CO_2 than serum obtained from the same blood from which the CO_2 has not been abstracted, thus:

	A	*B*
Serum from normal blood (alveolated)	33 % CO_2	46·3 % CO_2
Serum from CO_2-free blood (alveolated)	22 % „	20 % „
Diminution in CO_2 capacity	11 % „	26·3 % „

The diminished capacity of serum obtained from CO_2-free blood to carry carbon dioxide, although the ash of this serum contains just as much alkali as the ash of serum from normal blood (*vide supra*) might be due to the production of lactic acid in blood freed from CO_2 combining with the sodium bicarbonate and so rendering the sodium unavailable for carrying CO_2. The plausibility of this suggestion is indicated by the following lactic acid determinations:

	CO_2 capacity	% of lactic acid
Serum from normal blood (alveolated)	33 %	·1 %
Serum from same blood after freeing from CO_2	22 %	·173 %

Experimentally, *in vitro*, ·073 p.c. lactic acid, if entirely combined with $NaHCO_3$, diminishes the carbon dioxide carrying power of a fluid to which it is added 18·4 p.c. In the above experiment the increase in the lactic acid content of blood to this extent diminished the CO_2 capacity of the serum by 11 p.c. only. The production of lactic acid in blood in which the hæmoglobin is saturated with oxygen brings this phenomenon into opposition with the results observed by Fletcher and Hopkins in frog's muscle.

The production of lactic acid in blood affords an explanation of the more ready removal of CO_2 from the blood than from serum, and incidentally shows that the corpuscles constitute the source of the lactic acid. In every sample of cat's blood investigated the entire CO_2 was removed by CO_2-free air. In the case of serum CO_2 could not be removed by CO_2-free air. The following figures illustrate the effect of CO_2-free air on serum:

Serum (alveolated)	37·1 % CO_2	
Serum (air free from CO_2 for four hours) (*)...	7·6 % ,,	(residual CO_2)
(*) alveolated	33 % ,,	

These figures show two facts: (i) CO_2 cannot be removed from serum by CO_2-free air, and (ii) after the removal of as much CO_2 as possible, the serum can be re-alveolated to practically the same value as before. These facts correspond with those observed in the production of lactic acid in serum. Serum, from which all the corpuscles have been removed, does not produce lactic acid. This results in (i) a considerable quantity of CO_2 remaining in the serum as Na_2CO_3 after air free from CO_2 has been pulled through it for four hours, and (ii) the serum after such treatment being able to recombine with practically as much CO_2 as the original serum. Further, the results show that the corpuscles give rise to the lactic acid produced in shed blood. The origin of lactic acid in shed blood from corpuscles affords a simple explanation of the phenomena originally observed by Pflüger, that the entire CO_2 can be removed from blood but that with serum this result cannot be attained. Incidentally the ultimate substance which acts as an acid in blood which is not found in serum is not the corpuscles as suggested by Pflüger, nor the hæmoglobin as has been assumed at a later date, but the lactic acid produced by the formed elements of the blood. On the assumption that serum exposed to CO_2-free air for four hours contains Na_2CO_3 only the above figures show that the serum under consideration contained 15·2 p.c. CO_2 in combination with Na as $NaHCO_3$ and 22 p.c. CO_2 combined with the proteins. The figures given by Loewy are 18·8 CO_2 with the bicarbonate system and 11·8 p.c. CO_2 with the colloidal indiffusible substances in the blood.

(iii) The effect of adding lactic acid to blood on its capacity to carry carbon dioxide. The above results, illustrating the formation of lactic acid in shed blood and the change in the CO_2 capacity of such blood, suggested that the addition of lactic acid would not diminish the capacity of blood to carry carbon dioxide to such a degree as would be demanded by the bicarbonate hypothesis. To test this assumption varying quantities of lactic acid were added to fresh defibrinated blood and the CO_2 capacities of the sera obtained from the various fractions determined. The following results were obtained:

	Amount of lactic acid added to original blood	CO_2 capacity of serum
Fresh blood	0	33 % CO_2
" "	·1 %	27·3 % "
" "	·2 %	18·0 % "
" "	·3 %	13·0 % "

As previously stated, the addition of ·1 p.c. lactic acid to blood on the bicarbonate hypothesis should diminish its capacity to carry CO_2 approximately 24 p.c. From the figures it is evident that no such change in the CO_2 capacity of the serum was observed. Therefore the lactic acid must have united not with the alkaline salts only but with some other constituent of the blood, presumably the protein. The nature of this combination of the lactic acid and the protein is essentially labile since after removing the cells and protein from the blood the quantities of lactate radical present in the filtrates were as follows:

	Lactic acid
Original fresh blood to which no lactic acid had been added ...	·1 %
Blood to which ·1 % lactic acid had been added...	·2 %
Blood to which ·2 % lactic acid had been added...	·306 %
Blood to which ·3 % lactic acid had been added...	·4 %

Allowing for ·1 p.c. lactic acid contained in the original blood it is evident that no added lactic acid was removed by the corpuscles or coagulated protein. On the bicarbonate hypothesis the addition of ·13 p.c. lactic acid to the blood should have neutralised all the bicarbonate and have prevented the serum from carrying any CO_2 except in solution. The discrepancy between the results demanded by the bicarbonate hypothesis and the results actually obtained are evident from the above figures. Incidentally they show one or both of two things, (a) either CO_2 is carried other than as sodium bicarbonate, or (b) lactic acid added to blood is not neutralised by bicarbonate only but by other substances in blood, *i.e.* protein. Both these possibilities ultimately lead to the same conclusion—that the protein of blood can combine with acids, lactic acid or carbonic acid, and that the resulting complex is of an essentially labile nature.

The capacity of proteins of blood to carry carbon dioxide.

(i) Fibrinogen. Many phenomena observed in the coagulation of blood indicate that carbon dioxide may enter into intimate relations with fibrinogen. Among such phenomena may be mentioned (a) the coagulation of peptone blood by carbon dioxide, (b) the adjuvant effect of carbon dioxide on the production of intravascular coagulation by the injection of thrombokinase, and (c) the titratable alkalinity of blood is diminished after coagulation (Loewy and Zuntz; v. Limbech). A series of experiments were therefore carried out to determine the effect of coagulation on the alkali reserve of blood, plasma, and fibrinogen.

The blood of an anæsthetised cat was obtained directly from the carotid artery. To a portion of it potassium oxalate was added to the extent of ·1 p.c. A second portion was allowed to clot and the fibrinogen was removed by whipping. The oxalated blood and the defibrinated blood were then alveolated and their CO_2 content determined.

Oxalated blood	58 % CO_2
Defibrinated blood	53·6 % „

A control experiment showed that ·1 p.c. potassium oxalate had no effect on the capacity of blood to carry carbon dioxide. The capacity of blood to combine with carbon dioxide was definitely diminished by the coagulation and removal of fibrin.

An experiment similar to the above was carried out on plasma. Potassium oxalate to the extent of ·1 p.c. was added to the blood of a cat and oxalate plasma obtained by centrifuging. A portion of this plasma was clotted by the addition of a trace of fibrin ferment and the serum obtained after removal of the coagulum. The carbon dioxide contents of the plasma and serum were compared after both fluids had been exposed to alveolar air.

Oxalated plasma	51·1 % CO_2
Serum obtained from plasma ...	44·0 % „

These results, also, show that the capacity of the fluid of blood to carry carbon dioxide is diminished by the coagulation and removal of fibrin.

This series of experiments was completed by determining the capacity of a solution of fibrinogen to carry carbon dioxide. Fibrinogen was obtained from oxalated plasma by a method which has been previously described by one of us(16). 25 c.c. of plasma, obtained from oxalated ox blood, were added to 225 c.c. of distilled water, and the resulting fluid brought to the isoelectric point for globulin by the cautious addition of ·1 p.c. acetic acid. The precipitate of globulin was allowed to settle and then obtained as a compact mass by centrifuging. The fibrinogen was dissolved in 25 c.c. of NaCl 1 p.c. forming a clear solution which readily coagulated on the addition of fibrin ferment. The capacities of the fibrinogen solution and of the fluid remaining after coagulation of the fibrinogen and removal of the clot to combine with carbon dioxide were determined.

Fibrinogen in 1 % NaCl (alveolated)	9 % CO_2
Fluid after removal of fibrin (alveolated) ...	3 % „

Whatever view is taken as regards the globulin of plasma, *i.e.* whether it is regarded as consisting of fibrinogen only which on coagulation splits into fibrin and serum globulin, or whether fibrinogen and serum globulin are considered to be present in plasma, it is clear that fibrinogen dissolved in neutral salt solution combines with carbon dioxide. On the assumption that the only globulin present in plasma is fibrinogen, and taking the amount present in ox plasma as ·5 p.c., the above figures show that a gram molecular weight of carbon dioxide is carried by approximately 1250 grams of fibrinogen. The amount of fibrinogen which dissolves in a gram molecular weight of acid or alkali is approximately 16,000 grams. It is necessary to assume therefore that the association of carbon dioxide with fibrinogen is not of the same kind as that involved in the solution of globulin by acids and alkalies. The results indicate that carbon dioxide is adsorbed by fibrinogen rather than that a chemical relation exists between the two substances.

(ii) Serum protein from normal blood. The experimental results indicating that carbon dioxide may be associated with fibrinogen demanded that a similar investigation should be made on the protein of serum. The initial difficulty was to obtain the proteins of serum free from salt without decomposing the labile protein complex. Bayliss(8) freed serum from salt by dialysis and found that the resultant fluid possessed no capacity to carry carbon dioxide. The objection to this method is the prolonged dialysis which is necessary to free serum from diffusible substances. Precipitation of the proteins by neutral salts also appeared to be inadmissible owing to the large quantity of neutral salt required, and the difficulty of freeing the precipitate from the precipitating salt. A method previously described by one of us(17) of precipitating the serum by cold alcohol was adopted. This method consists in cooling absolute alcohol to – 10° C. by a mixture of ice and salt and adding to the cooled alcohol an equal volume of serum cooled to 0° C. The mixture is kept at – 10° C. for ten minutes after which the precipitated protein is obtained as a compact mass by a Martin's centrifuge. The precipitate is washed once with absolute alcohol and then twice with ether. After this it is air-dried and dissolved in water. The protein dissolves readily in water forming a solution very similar in appearance to that of the original serum. As evidence of the small amount of change suffered by the proteins in this procedure it may be stated that if plasma be used as the original fluid the solution obtained after alcohol precipitation, etc., coagulates on adding a trace of fibrin ferment. The protein precipitated from human serum by cold alcohol, dissolved in a volume of water equal to that of the original serum, combined with 21·3 p.c. CO_2 when exposed to alveolar air. This protein was associated with a considerable quantity of inorganic salts. To determine the relative quantities of carbon dioxide associated with the protein and the salts contained in the precipitated complex a similar experiment was done on another sample of the same serum in which the protein precipitate was ashed. The ash was dissolved in a volume of water equal to that of the original serum. This fluid combined with 15·5 p.c. CO_2. Therefore, assuming that all the alkali contained in the ash capable of carrying carbon dioxide existed in the protein precipitate in an equally free manner, the figures show that 5·8 p.c. CO_2 was associated with the protein independently of any alkaline salt which might have been contained in its complex. Incidentally the results show that sodium bicarbonate does not exist free in serum since 50 p.c. alcohol at – 10° C. does not precipitate a ·2 p.c. solution of sodium bicarbonate. Evidently the alkaline salts contained in the protein precipitate were present in the serum associated with the protein complex. The above experiment has been repeated with many sera. The following figures illustrate the results obtained from cats' serum:

Serum protein	22·5 % CO_2
Ash of protein	11·0 % ,,
Minimum quantity of CO_2 associated with protein	11·5 % ,,

The experimental results indicate that not only fibrinogen, but also the other proteins of plasma combine with carbon dioxide.

(iii) Serum protein obtained from serum saturated with carbon dioxide and serum freed from carbon dioxide. On the hypothesis that serum protein acts as a weak acid and thereby confers on sodium bicarbonate the function of being an efficient physiological carrier of carbon dioxide it is clear that protein obtained from serum saturated with CO_2 should contain the minimal number of sodium ions in its complex whilst protein obtained from serum freed from CO_2 should be fully loaded with sodium ions. In the former case such protein dissolved in water should possess a capacity to carry carbon dioxide much greater than that of protein obtained from CO_2-free serum. To test this hypothesis two portions of the same serum were taken. The first portion was saturated with carbon dioxide. After saturation it was found to contain 152 p.c. of carbon dioxide. The second portion had CO_2-free air pulled through it for four hours. At the end of that time its CO_2 content was reduced to 8 p.c. Both these serum fractions were precipitated by 50 p.c. alcohol at – 10° C., and the protein precipitated from each was dried, in the way previously described. In each case the dried precipitate was dissolved in a volume of water equal to that of the original serum. The quantities of CO_2 contained in each of these solutions after being submitted to alveolar air were now determined with the following results: Protein precipitated from serum saturated with CO_2 combined with 18·4 p.c. CO_2 when dissolved in a volume of water equal to that of the original serum. Protein precipitated from serum partially freed from CO_2 when dissolved in a volume of water equal to that of the original serum combined with 17·4 p.c. CO_2.

The practical identity of the capacity to carry carbon dioxide of protein obtained from (*a*) serum saturated with CO_2 and (*b*) serum freed from CO_2 indicates that proteins do not function as acids in the bicarbonate system of serum and that the amount of sodium contained in the protein complex does not vary with the quantity of CO_2 contained in the serum.

REMARKS.

Direct evidence against the hypothesis that hæmoglobin or the proteins of blood function as weak acids capable of sharing the available alkali of blood with CO_2 has been given in the foregoing pages. This evidence consists of (*a*) the equality of the available alkali contained in the ash of serum obtained from CO_2-free blood and normal blood, (*b*) the equality of the available alkali contained in the ash of protein precipitated from CO_2-free serum and normal serum, and (*c*) the effect of adding lactic acid to blood. These experimental results extend the observations of **Buckmaster**(18), who found that CO_2 cannot be liberated from $NaHCO_3$ or Na_2CO_3 by hæmoglobin or any constituent of defibrinated blood, or dialysed laked blood. The results indicate that the association of CO_2 with protein is essentially labile and of the nature of adsorption.

We may add some remarks on the arguments in favour of the bicarbonate hypothesis based upon the assumption that hæmoglobin can function as an acid in blood. (i) The isoelectric point for hæmoglobin is lower than the P_H of blood. But if the isoelectric points of other substances in blood be considered it is found that the proteins and amino acids of plasma are relatively more acidic than oxyhæmoglobin. On this argument the proteins of plasma would occupy a stronger position in the bicarbonate hypothesis than hæmoglobin. (ii) Blood, but not serum, can be freed from CO_2 by exposure to a vacuum. The experiments recorded indicate that the production of lactic acid by the corpuscles of blood is the main factor in expelling the entire CO_2 from blood when exposed to a vacuum. (iii) The oxidation of blood results in the evolution of CO_2 and increases its hydrogen ion concentration. The assumptions that the increased acidity of blood on oxidation is due to changes in the hæmoglobin, and that the evolution of CO_2 is due to this cause are not founded on experiments in which the only substances involved are hæmoglobin, oxygen and sodium bicarbonate. The peroxidase capacity of hæmoglobin, the production of lactic acid in cells in the absence of oxygen, the oxidative removal of lactic acid, the influence of the gases of the blood on the permeability of the red cells are factors which might produce the effects observed.

SUMMARY.

(1) Lactic acid is produced from the corpuscles in shed blood. This explains: (*a*) the steady fall in the CO_2 of shed blood; (*b*) the power to abstract CO_2 from blood and not from serum by a CO_2 vacuum, and (*c*) the diminished capacity of blood, from which CO_2 has been removed, to recombine with CO_2.

(2) The addition of lactic acid to blood *in vitro* diminishes its capacity to carry CO_2 by less than 50 p.c. of the quantity demanded by the bicarbonate hypothesis. The association of lactic acid with protein is essentially labile since on coagulation of the protein all the lactate radicle is present in the filtrate.

(3) Solutions of the ash of sera obtained from (*a*) normal blood, and

(*b*) the same blood freed from CO_2 combine with equal volumes of CO_2. There is no evidence that any ionic interchange takes place between the corpuscles and serum on freeing blood from CO_2.

(4) Solutions of serum proteins obtained from (*a*) serum freed from CO_2, and (*b*) serum saturated with CO_2 combine with the same volume of CO_2. There is no evidence that the proteins of serum associate with Na when CO_2 is removed from blood.

(5) The coagulation of fibrinogen contained in any fluid—blood, plasma or a fibrinogen solution—diminishes the capacity of that fluid to carry CO_2. Quantitative results indicate that the association of CO_2 with fibrinogen is of the nature of adsorption.

(6) Solutions of protein obtained from serum by alcohol precipitation combine with more CO_2 than can be calculated as existing in combination with the alkaline salts contained in the precipitated complex.

(7) These results indicate: (*a*) that the bicarbonate hypothesis is inadmissible since neither hæmoglobin nor serum proteins function in blood as weak acids sharing the available Na with the CO_2 according to the requirements of the organism; (*b*) that the proteins of plasma adsorb CO_2 which in the case of fibrinogen is relatively large in amount; (*c*) that CO_2 is combined in blood in two ways, (i) adsorbed by the proteins and (ii) as $NaHCO_3$; (*d*) that the transport of CO_2 is effected by the protein; and (*e*) that the alkali reserve of blood is constituted by (i) $NaHCO_3$ and (ii) protein.

(The expenses of this research have been defrayed by a grant from the Royal Society.)

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THE RELATION BETWEEN RESPIRATION AND THE PULSE-RATE.

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It is well known that a close relationship usually (though not invariably) exists between the degree of activity of the respiratory centre and the rate of the pulse. In man, for example, the dyspnœa induced by breathing air containing a slight excess of carbonic acid is often associated with considerable acceleration of the pulse-rate. Several explanations of this relationship have been put forward. Traube suggested that, when the respiratory centre is unusually active, an overflow or irradiation of impulses from the respiratory to the cardio-inhibitory centre takes place and that these impulses lessen the tone of the cardio-inhibitory centre. This view was supported by the observation of Frédéricq that, when the chest was open and the lungs collapsed, the frequency of the pulse varied synchronously with the respiratory efforts made by the animal. On the other hand, Hering's observations led him to conclude that afferent impulses passing from the lungs to the cardio-inhibitory centre reflexly alter the pulse-rate, and that such impulses, set up by distension or collapse of the lungs, are responsible for the inspiratory quickening and expiratory slowing of the pulse often observed in animals. This conclusion was accepted by Brodie who found that stimulation of the central end of the pulmonary branches of the vagi caused inhibition of the heart. Another possibility is that increased respiratory movements influence the pulse-rate mainly or entirely by increasing the return of blood to the heart, thereby evoking reflex acceleration of the heart.

The experiments described in this paper were carried out in order to determine first how far each of these explanations holds good and, second, to what extent the respiratory system is concerned in the regulation of the pulse-rate.

Methods. Almost all the experiments were carried out on cats anæsthetised with ether; a few observations were made on dogs anæsthetised with chloroform and ether after a preliminary dose of morphia. The chest was widely opened and the phrenic nerves cut in order to exclude as far as possible the mechanical influence of the respiratory movements on the circulation. Artificial respiration was maintained by means of a pump, and the degree of ventilation of the lungs was usually recorded by connecting a side tube on the tracheal cannula with a recording tambour. The composition of the air blown into the lungs could be varied by attaching a bag containing the desired mixture of gases to the inlet tube of the respiration pump. The respiratory movements made by the animals were recorded by attaching a thread to the chest wall; the other end of the thread was attached to a recording lever or tambour. As a rule, either the stellate ganglia were removed or the cardiac accelerated nerves were divided.

Except where otherwise stated the upper tracing in each figure represents the respiratory movements of the chest wall, the middle tracing indicates the blast of the respiration pump, and the lower tracing records the arterial pressure. The time marker records periods of ten seconds. The numbers placed either below the blood-pressure tracing or just beneath the record of the respiratory pump indicate the pulse-rate at that moment.

RESULTS.

The observations to be described fall into two main groups. The first consists of experiments relating to the possibility of irradiation of impulses from the respiratory to the vagus centre; the second includes experiments carried out to determine whether distension and collapse of the lungs gives rise to afferent impulses travelling up the vagi to the vagus centre, and reflexly modifying its tone.

Irradiation from the respiratory centre. The activity of the respiratory centre was excited, or increased, by raising the tension of carbonic acid in the blood, and several methods were adopted for this purpose. In some experiments the blast of the respiration pump was kept constant, and a small amount of carbonic acid was added to the air blown into the lungs (Fig. 1). In others, in confirmation of experiments previously described (2), the blast of the pump was reduced so as to cause very slight inflation of the lungs, oxygenation of the blood being maintained by blowing into the lungs air containing a very large percentage of oxygen. In other experiments again the artificial respiration was almost stopped for a short time (Fig. 2).

Although each of these procedures led to a striking increase in the activity of the respiratory centre, as measured by the respiratory movements of the chest-wall, the pulse-rate was unaffected for a time, as is seen in Figs. 1 and 2; as the respiratory efforts became increasingly violent, marked slowing of the pulse took place. It was necessary, when carbonic acid was being blown into the lungs, to keep its percentage very low; if a large percentage of carbonic acid was blown into the lungs, the respiratory movements at once became very violent and the pulse-rate immediately became very slow. In all the experiments just described the presence of vagus tone, and therefore the possibility of acceleration of the pulse-rate, was demonstrated either by subsequent section of the vagi or by other means. Apart from the experiments in which asphyxia was produced, the arterial pressure and the pulse-pressure remained almost steady, and no evidence was obtained that the respiratory

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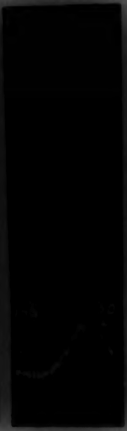

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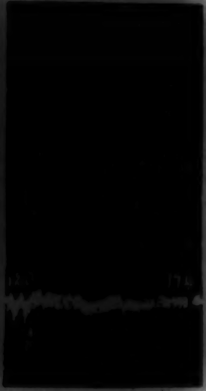

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Fig. 1. Effect of adding CO_2 to the air entering the lungs. Top tracing = resp. efforts, B.P. 90–95 mm. Hg.

Fig. 2. Effect of asphyxia. Top tracing = resp. efforts. B.P. at onset of asphyxia = 94 mm. Hg.

Fig. 3. At A the blast of the pump was gradually increased. B.P. = 110 to 115 mm Hg.

efforts made by the animal appreciably affected the return of blood to the heart unless these efforts became almost convulsive in character.

These experiments show that, when the influence of the respiratory movements on the circulation is excluded, even marked and exaggerated activity of the respiratory centre, such as would normally manifest itself as severe dyspnœa, does not give rise to an overflow or irradiation of impulses to the cardio-inhibitory centre of such a kind as to lessen the tone of this centre. The cause of the slowing of the pulse-rate associated with very violent respiratory efforts will be discussed subsequently.

Afferent impulses from the lungs. In confirmation of Yandell Henderson's observation it was found that vigorous artificial ventilation of the lungs invariably brought about considerable acceleration of the pulse-rate and abolished the activity of the respiratory centre (Fig. 3).

The suggestion that the over-ventilation of the lungs mechanically interferes with the flow of blood through the lungs, and that the acceleration of the pulse is secondary to the changes in the circulation naturally presents itself in the first instance. Although excessive pulmonary ventilation undoubtedly obstructs the flow of blood through the lungs, it is possible, when the chest is opened and the lungs can expand freely, to carry out fairly vigorous artificial respiration and yet to avoid any material disturbance of the circulation. If the blast of the pump is carefully regulated the lungs can be considerably over-ventilated without producing any appreciable change in the arterial pressure and the pulse pressure, and with a comparatively small rise in the venous pressure. In a few experiments the pressure in the superior vena cava was noted, and it was found that even fairly vigorous artificial respiration raised the pressure by only a few mm. of water. It is probable, therefore, that mechanical interference with the circulation in these experiments plays a comparatively subsidiary part in bringing about the acceleration of the pulse, though its influence cannot always be entirely excluded.

Apart from the mechanical factor, there are two possible explanations of the acceleration of the pulse brought about by over-ventilation of the lungs. The first is that the acceleration may be reflex in origin and dependent on the presence in the lungs of afferent fibres travelling up the vagus to the cardio-inhibitory centre, and that distension of the lungs sets up impulses which pass along these fibres and reflexly lessen the tone of the cardio-inhibitory centre. The second is that the alteration in the reaction of the blood, consequent on the over-ventilation of the lungs, directly affects the tone of the cardio-inhibitory centre.

For the purpose of deciding between these two possibilities, Brodie and Russell's experiments on the effect of stimulating the central end of the pulmonary branches of the vagi were repeated. These observers exposed the pulmonary vagi by resecting the sixth to the ninth ribs from the sternum to the vertebral column, usually on one side, in the dog. Stimulation of the different branches passing from the lungs to the vagus trunk led to slowing of the heart and inhibition of respiration; and comparatively weak stimuli were effective. In the present enquiry six experiments have been made on this point, four in cats and two in dogs; the chest was widely opened and the pulmonary nerves were exposed as they left the vagi to enter the lungs. On the left side the pulmonary branches leave the main vagus trunk just at or below the top of the aortic arch; on the right side they leave the vagus below the azygos vein. In the dog the nerves are sufficiently large to be dissected out and stimulated separately. The nerves in the cat are so slender that a different procedure was adopted. The vagus was divided below the lungs, and the central end of the vagus was stimulated just above the point at which the pulmonary fibres joined it and below the point at which the lowest cardiac fibres left the vagus trunk; for this purpose a

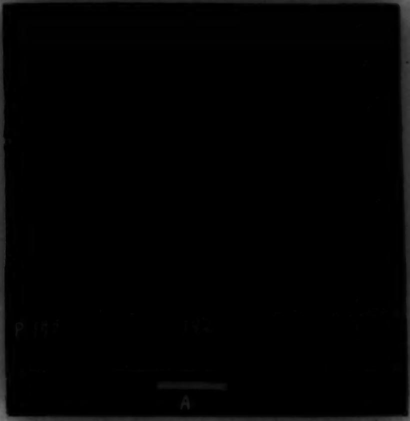

Fig. 4. Dog. Upper tracing = resp. efforts. B.P. 105 mm. Hg. At A the central end of the left pulmonary vagus was stimulated with a fairly strong current.

sufficient length of the vagus trunk is available, especially on the right side. In every experiment the pulmonary vagal branches were stimulated on both sides.

In the dog, stimulation of the central end of the pulmonary branches of the vagi caused absolutely no change in the pulse-rate, although the respiratory movements were completely inhibited (Fig. 4). The result

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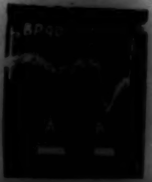

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Fig. 5. Cat. B.P. = 100 mm. Hg. Stimulation of right pulmonary vagus (central end). Upper tracing represents respiratory movements of the chest-wall.

Fig. 6. Cat. A, stimulation of right pulmonary vagus low down. B, electrodes moved up nearer the cardiac branches of the vagus.

Fig. 7. A, stimulation of left pulmonary vagus (central end). B, stimulation of left vagus (central end) in the neck.

was negative whatever the strength of current used. In the cat also, stimulation of the vagus just above the point at which the pulmonary fibres joined it, while inhibiting respiration, had no effect on the pulse-rate (Fig. 5), provided that care was taken not to use a very strong current and not to apply the electrodes too near the point at which the cardiac nerves leave the main vagus trunk; in the latter case some slowing of the heart took place and was presumably due to escape of current. For example, in the experiment illustrated in Fig. 6 stimulation of the right pulmonary vagus as low down as possible (A) had no effect on the pulse-rate; when the electrodes were moved up nearer to the cardiac fibres inhibition of the heart took place (B). That the reflex path for cardiac inhibition had not been disturbed during the operative procedure involved in the experiments was always demonstrated either by subsequently cutting one vagus in the neck and stimulating its central end (Fig. 7 B) or by applying the electrodes to the central end of the cardiac branches of one vagus.

Some experiments were also made to determine the effect on the pulse-rate of over-ventilation of the lungs after section of the pulmonary branches of the vagi. It is obvious that, if the acceleration of the pulse is brought about by afferent impulses passing from the distended lungs to the cardio-inhibitory centre and lessening its tone, the effect should be abolished by cutting the pulmonary branches of the vagi. The experiments showed, however, that vigorous artificial respiration produced acceleration of the pulse equally well whether the pulmonary branches of the vagi were intact or had been divided. The result of a typical experiment is shown in the following protocol:

Cat; ether anæsthesia. Chest widely open and phrenic nerves divided; artificial respiration.

			Blast of pump	
			moderate	vigorous
(1) Vagi intact	Pulse-rate	(a)	144	192
		(b)	128	180
(2) Pulmonary branches of vagi divided	Pulse-rate	(a)	120	160
		(b)	120	180

The evidence just detailed definitely points to the conclusion that no afferent fibres pass (in the vagi) from the lungs to the cardio-inhibitory centre, and that, consequently, distension or deflation of the lungs does not give rise to impulses capable of reflexly bringing about an alteration in the pulse-rate.

The observation that vigorous artificial respiration leads to acceleration of the pulse-rate even after section of the pulmonary vagi suggests that the altered reaction of the blood, consequent on the washing out of carbonic acid from the blood, is responsible for the increased pulse-rate. Direct evidence in favour of this view was furnished by experiments in which vigorous artificial respiration was carried out, a small amount of carbonic acid being added to the air blown into the lungs. The amount of carbonic acid sent into the lungs was so adjusted as to compensate approximately for the over-ventilation and thus to prevent the washing out of carbonic acid from the blood. Under these conditions, the activity of the respiratory centre and presumably the reaction of the blood were unaffected by the over-ventilation of the lungs. After a short time the blast of the pump was reduced and atmospheric air was once more blown into the lungs. The pulse-rate remained the same before, during, and after, the period of over-ventilation. This is shown in Fig. 8 which represents the transition from vigorous artificial respiration, carbonic acid being added to the air, to moderate artificial respiration with atmospheric air. The respiratory movements of the chest wall gave a poor graphic record, but direct observation of these movements showed that they were practically the same whether the blast of the pump was moderate or strong.

Fig. 8. Upper tracing = respiratory efforts. At A the blast of the pump was reduced, and air free from CO_2 was blown into the lungs. Previous to the point A the air entering the lungs contained carbonic acid.

This experiment makes it clear that neither distension of the lungs as such, nor mechanical disturbance of the circulation due to the distension, plays any significant part in bringing about the acceleration of the pulse usually associated with over-ventilation of the lungs, and that the important factor at work is the abolition of the activity of the respiratory centre consequent on a fall in the H ion concentration of the blood.

Hill and **Flack** have shown that the cardio-inhibitory centre is stimulated by a sudden rise in the tension of carbonic acid in the blood, and **Langley** has called attention to the readiness with which the centre responds to this stimulus. These observations have been repeatedly confirmed in the present enquiry. When the lungs are being artificially ventilated and the blast is constant, the addition of carbonic acid to the air entering the lungs must bring about a fall in the p_H of the blood, since there is no compensatory action on the part of the respiratory system. The intensity of the respiratory efforts made by the animal in these circumstances was taken as evidence of such a change, and no attempt was made to measure the p_H of the blood directly. Similarly the abolition of the activity of the respiratory centre as a result of over-ventilation of the lungs was assumed to be due to a rise of the p_H of the blood. There can be little doubt, therefore, that the tone of the cardio-inhibitory centre is directly affected by the reaction of the blood, being intensified by a fall, and lessened or abolished by a rise, in the p_H of the blood. It does not seem very probable, however, that variations in the p_H of the blood can play any effective part in regulating the pulse-rate in the normal individual, since all the evidence goes to show that, except during muscular exercise, the reaction of the blood is practically constant; and during exercise other factors affecting the pulse-rate override the influence of the altered p_H of the blood.

The observations just described lead to the conclusion that neither the degree of activity of the respiratory centre nor the degree of distension of the lungs have in themselves any influence on the pulse-rate either by irradiation to the cardio-inhibitory centre, or by setting up a reflex alteration of the tone of this centre. The cause of the acceleration of the pulse usually observed in the normal animal or in man during hyperpnœa or dyspnœa must be sought, therefore, in the changes taking place in the circulation as a result of the respiratory movements. It appears necessary also to regard changes in the circulation as the most probable explanation of the inspiratory acceleration and expiratory slowing of the pulse-rate which, when it occurs in man, is known as sinus arrhythmia. Further, it seems clear that, since the respiratory system does not affect the pulse-rate either by irradiation or by afferent impulses from the lungs, the rate of the pulse must be controlled primarily by the heart itself. The work of Krogh and of Evans has made it evident that, generally speaking, the efficient and economical working of the heart necessitates a close correlation between the pulse-rate and the work done by the heart; and it is essential for this purpose that the heart should possess some means whereby it can regulate the frequency of the pulse. On this view, the variations in the pulse-rate during hyperpnœa or in sinus arrhythmia represent one aspect of a fundamental process concerned in regulating the pulse-rate under all conditions.

The means available are first the depressor nerve and, second, the reflex path whereby diastolic distension of the right side of the heart evokes acceleration of the pulse.

It appears highly probable that the acceleration of the pulse-rate associated with dyspnœa is due to the fact that the more vigorous respiratory movements lead to a larger return of blood to the heart; this, by bringing about some diastolic distension of the heart gives rise to reflex acceleration of the pulse. The presence of this reflex mechanism also furnishes a satisfactory explanation of rhythmic inspiratory acceleration and expiratory slowing of the pulse-rate (sinus arrhythmia).

Each inspiration increases the diastolic filling of the heart and gives rise to reflex acceleration of the pulse-rate; with the onset of expiration the stimulus to acceleration passes off and the pulse becomes less frequent. The conditions predisposing to sinus arrhythmia appear to be an infrequent pulse and slow deep breathing. In these circumstances, the diastolic filling of the heart will be greater than usual and the accelerator reflex will be more readily evoked.

On the other hand, it is possible that both the cardiac depressor reflex and the accelerator reflex are involved in the rhythmic variations of the pulse-rate during respiration, and that the occurrence of these rhythmic changes in pulse-rate is due to a balancing action on the part of these two opposing reflexes. On this view, the greater diastolic filling of the heart during inspiration leads to reflex quickening of the pulse-rate; at the same time the output of the heart increases and the arterial pressure rises. The rising arterial pressure brings the depressor reflex into action, and the pulse slows during expiration, partly for this reason and partly because the stimulus to acceleration passes off. If this explanation is correct, the normal steady rate of the pulse must be due either to a more perfect balancing of the depressor and accelerator reflexes or to their not being called into action.

An attempt was made to ascertain whether, under suitable conditions, both the accelerator and the inhibitory cardiac reflexes are simultaneously evoked. The abdomen was compressed before and after section of the left vagus. The heart was enclosed in a cardiometer and its volume and output were graphically recorded. It was found that the increased volume and output of the heart, and the raised arterial pressure, consequent on the abdominal compression, led to slowing of the pulse-rate when both vagi were intact, and in some experiments to acceleration of the pulse after section of the left vagus. The following protocol illustrates an experiment of this kind:

Cat anæsthetised with ether. Heart enclosed in cardiometer. Artificial respiration.

	Arterial pressure	Pulse-rate	Volume and output of heart
(*a*) Both vagi intact.			
(1) Before abd. compression	24	156	—
(2) During " "	44	132	Vol. larger; output per beat almost doubled.
(*b*) Left vagus divided.			
(1) Before abd. compression	32	156	—
(2) During " "	52	168	Vol. larger; output per beat increased.

The result just described was not invariably obtained and, as the outcome of a large number of experiments on this point, it seems probable that considerable variation exists in the relative proportion of afferent cardiac fibres running in the right and left vagus respectively. But it appears that, at least in some cats, the cardiac depressor fibres which carry impulses bringing about reflex slowing of the pulse-rate run mainly, though not entirely, in the left vagus (including the depressor nerve). In such cases section of the left vagus makes it possible to bring about acceleration of the heart by abdominal compression. These experiments suggest, therefore, that, when the diastolic volume of the heart and the arterial pressure are simultaneously increased, both the accelerator and the inhibitory cardiac reflexes are set in action, although the inhibitory reflex is the more powerful.

In the present enquiry no definite evidence was obtained to show whether these reflexes are constantly in action or are only evoked when the arterial pressure or the diastolic distension of the right side of the heart rises above the normal level; but it is evident that, if they are not usually in action the cardio-inhibitory centre must possess an inherent tone susceptible of modification by these reflexes, whereas, if these reflexes are constantly at work, the tone of the cardio-inhibitory centre must be largely or even wholly reflex in origin. This point is being investigated.

CONCLUSIONS.

(1) No evidence was obtained either that impulses pass by irradiation from the respiratory centre to the cardio-inhibitory centre and alter the tone of the latter or that afferent impulses, capable of reflexly affecting the pulse-rate, pass from the lungs to the cardio-inhibitory centre.

(2) The tone of the cardio-inhibitory centre is modified by changes in the reaction of the blood, being increased by a fall, and diminished by a rise, in the p_H of the blood.

(3) The regulation of the pulse-rate is carried out primarily by the heart itself, and the respiratory movements influence the pulse-rate only in so far as, by bringing about changes in the circulation, they call into action the regulative mechanism possessed by the heart.

This investigation was carried out with the aid of a grant from the Medical Research Committee to which I wish to express my indebtedness.

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THE INFLUENCE OF VENOUS PRESSURE UPON THE HEART-RATE.

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Bainbridge(1) found that increased venous filling of the heart in dogs by injection of blood or saline solution, led to a rise of venous pressure and to acceleration of the heart. He concluded that this quickening of the heart rate is reflex in origin and is due chiefly to diminution of vagus tone and partly to increased accelerator tone, since it is not necessarily or usually accompanied by changes in arterial pressure or in the respiratory movements, and it does not depend upon the character and amount of the injected fluid, except in so far as this raises the venous pressure. He found also no evidence that the activity of the suprarenal glands increased.

Such a reflex must play a very important part in the circulation. It stands in contrast with the diminution of heart-rate caused by increase of arterial pressure. Prof. **Starling** suggested to us that we should determine whether a similar reflex occurs in the frog. As our experiments on the frog gave negative results—the explanation of which we shall give later—we repeated the experiments on the rabbit, cat and dog, and having in certain cases obtained positive results, we proceeded to investigate the parts of the heart and blood vessels from which the reflex could be obtained. It may be mentioned here that it is essential both in the frog and mammal for the injected fluid to be at the same temperature as the heart; observations on this point in the mamma have been made by **Knowlton** and **Starling**(2).

Experiments on frogs.

Methods. The brain of the frog was destroyed, and then the heart was exposed by opening the chest. A cannula attached to one limb of a three-way tube was inserted into the left carotid artery, the second limb being connected with a mercury manometer and the third with a Γ-shaped tube, which was used to keep the artery pressure constant by allowing the excess of fluid to flow out, the height of the tube being changed at will. The left v. cava ant. or sometimes v. cava post. was connected with a cannula which was attached to one limb of a four-way tube; the other three limbs were connected with a water manometer, a thermometer and a reservoir respectively. Care was taken to avoid any mechanical injury of the vagus. By changing the height of the reservoir (Mariotte's bottle) the pressure under which Ringer's solution from it entered into the sinus was controlled. The heart-rate was sometimes counted by means of a stop-watch, sometimes the heart beats were recorded by Engelmann's method.

The preparation was left for some time before the beginning of the experiment, until the heart-rate became constant. The venous pressure was raised gradually or suddenly from zero or 1 cm. to a few or several cm. water pressure.

With this procedure the heart-rate instead of increasing decreased slightly. This decrease seemed to be due partly to the increase of arterial pressure, but chiefly to weakening of the heart, for it did not recover its original rate after the venous pressure was brought to the initial level. In some of our experiments, when the venous pressure was moderately raised, slight increase in the heart-rate was observed. This, however, was found to be due to the temperature of the heart being 1° C. or more below that of the Ringer's solution infused into the sinus, owing to evaporation from the exposed heart. In order to avoid this the arrangement in later experiments was modified by putting the whole heart preparation into a large glass bowl filled with Ringer's solution, the infused fluid being introduced into the sinus through a spiral tube immersed in the bowl. After this modification the heart-rate remained exactly the same in spite of rather considerable rise of venous pressure and dilatation of the heart.

Over one hundred frogs were used. The following protocols are typical examples.

1. *R. esculenta*; cardiac nerves intact. Goltz's reflex positive. Temp. 11° C.

	Pulse rate	Venous pressure	Arterial pressure
Before the experiment	24	0·5	3
Venous pressure raised	24	3	3
10′ later	24	0·5	3
20′ later	24	0·5	3

2. *R. esculenta*; cardiac nerves intact. Goltz's reflex positive. Temp. 13° C.

Before the experiment ...	30	1	4
Venous pressure raised ...	30	3	4
10′ later	30	1	4
20′ later	30	1	4

The absence of effect of venous pressure on the frog's heart may in large part be attributed to the normal absence of vagus tone, which many observers(3) have found in this animal. The experiments also show that venous pressure does not excite—or at any rate does not easily excite—sympathetic tone. Confirmation of this was obtained by repeating the experiment after injecting atropine; increased venous pressure had no effect on the heart-rate.

Experiments on warm-blooded animals.

Methods. The experiments were carried out on dogs, cats and rabbits anæsthetised with a mixture of chloroform and ether after a preliminary dose of morphia. The methods adopted were on the whole the same as Bainbridge's on dogs; some modifications were as follows: (1) the pulse-rate was counted from the tracing of the blood-pressure of the carotid artery. (2) In order to measure the venous pressure in most experiments the v. jug. ext. close to the pleural cavity instead of v. iliaca was connected with a water manometer. The fluid was usually rather rapidly injected in small or moderate amount into the v. jug. ext., for this method could be repeated several times on the same animal without inducing abnormal conditions of the circulation; but when the rate of heart was already quick owing to some cause (absence of vagus tone before or after atropine, etc.) larger amounts of fluid were injected. (3) The temperature of the animals was usually measured by inserting a thermometer through a small hole made in the second intercostal space into the mediastinal cavity; it rested upon the pericardium. (4) The rate at which the fluid was allowed to enter the vein was regulated by using a spring clip connected with the reservoir of the fluid, so that the arterial pressure was kept as nearly constant as possible during the injection. (5) The experiments were carried out under artificial respiration, so that the effect due to changes in respiration was excluded. (6) The venous pressure represented is not the absolute but the relative one.

Rabbits. The injection of a rather large amount of fluid for the size of this animal in spite of a considerable rise of venous pressure has no effect upon the heart-rate. The slight changes in the rate as shown in the protocols are more probably to be accounted for as unavoidable experimental errors than as a reflex action caused by the injection.

Of 16 cases of injection, 12 showed no change in heart-rate, 3 showed a slight increase, and 1 a slight decrease. If we had made a sufficient number of experiments we should probably have obtained an equal number of increasing and decreasing effects according to the law of errors. We consider then that in the rabbit increased venous pressure has no reflex effect on the heart-rate.

After dividing the vagus as well as after the administration of atropine we could not find any effect of venous pressure upon the heart-rate.

1. **Rabbit:** 1·2 kg. Temp. 39° C. Injection of 25 c.c. saline solution in 15″. Temp. 39° C.

	Pulse rate	Venous pressure	Arterial pressure
Just before injection	270	10	60
Just after injection ...	270	14	70
10″ after injection ...	270	11	65

2. **Belgian hare:** 2·3 kg. Temp. 39·3° C. Injection of 50 c.c. saline solution in 15″. Temp. 39° C.

Just before injection	288	10	100
Just after injection ...	288	18	105
15″ after injection ...	288	11·5	100

3. **Rabbit:** 1·5 kg. Temp. 37·5° C. Injection of 25 c.c. saline solution in 25″. Temp. 37·5° C.

Just before injection	222	9	65
At end of injection ...	220	15	65
10″ after injection ...	222	12	65
20″ after injection ...	222	10	65

The experimental results in the rabbit are in good agreement with those found on frogs. The rabbit is known to be an animal which has normally no vagus tone, especially when the lungs are well ventilated (4). Therefore our negative results are mainly due to the absence of vagus tone, and as in the frog the sympathetic effect is not easily produced by the rise of venous pressure.

Cats. Most cats have some vagus tone, and the rapid injection of moderate or larger amount of Ringer's solution evokes definite quickening. This will be seen from the following examples.

1. **Cat** (see Fig. 1): 3 kg. Temp. 37·5° C. Injection of 50 c.c. saline solution in 20″. Temp. 37·5° C.

	Pulse rate	Venous pressure	Arterial pressure
Just before injection	216	5·5	130
At end of injection ...	222	11	130
Just after injection...	240	17	130
15″ after injection ...	218	11	130
25″ after injection ...	216	10·5	130

2. **Cat:** 2·6 kg. Temp. 37·5° C. Injection of 50 c.c. saline solution in 25″. Temp. 37·5° C.

Just before injection	222	14·5	130
At end of injection ...	234	20	130
Just after injection...	246	26	130
20″ after injection ...	228	19	130
40″ after injection ...	222	17·5	130

The same effect can be produced after cutting off the stellate ganglion, while the vagus remains intact. From the following protocol it is seen that the magnitude of the changes in the heart-rate does not appreciably alter after cutting the ganglion,

Fig. 1. Showing the acceleration of heart-rate by injection of saline solution in cat.

3. Cat: 3·5 kg. Temp. 36° C. After section of stellate ganglion. Injection of 50 c.c. saline solution, in 20″. Temp. 36° C.

	Pulse rate	Venous pressure	Arterial pressure
Just before injection ...	144	8	110
At end of injection ...	150	10	115
Just after injection ...	156	20	110
10″ after injection ...	150	15	110
25″ after injection ...	144	10	110

In order to determine the reflex caused by increase of accelerator tone alone, we gave a dose of atropine sufficient to paralyse the efferent vagus endings, without affecting the fibres.

4. Cat: 3 kg. Temp. 38° C. Injection of 50 c.c. saline solution, in 20″. Temp. 38° C.

(*a*) Before injecting atropine.

	Pulse rate	Venous pressure	Arterial pressure
Just before injection ...	156	8	120
Just after injection ...	180	15	125
Later	156	9	120
(*b*) After injecting atropine.			
Just before injection ...	252	9	130
Just after injection ...	258	18	130
Later	252	11	130

All other cases yielded similar results, *i.e.* there was a slight, but only a slight, increase in the heart-rate. The increase we consider is a genuine effect due to the reflex acceleration.

When the vagi were divided, increased venous pressure had no effect on the heart-rate, showing that the normal effect is reflex and that the afferent fibres are in the vagus.

Dogs. Vagus tone in the dog is so marked that the heart-rate often becomes twice as quick or even more after the division of the vagi. Accordingly the acceleration produced by the injection of fluid causing a definite rise of venous pressure is usually far more marked in these animals than in cats.

Several experiments were made on dogs, and we obtained results like those of Bainbridge. The increase in heart-rate was obtained after section of the sympathetic, but not after section of the vagi. We failed to obtain unmistakable effects after the administration of atropine in small dogs, but we succeeded in finding it in dogs of a larger size. To compare the magnitude of the increase of heart-rate in dogs with that in other animals, the following two protocols are given.

1. Dog: 10 kg. Temp. 37·5° C.

(*a*) Injection of 100 c.c. saline solution, in 20″. Temp. 37·5° C.

	Pulse rate	Venous pressure	Arterial pressure
Just before injection ...	81	12	100
At end of injection ...	122	20	115
Just after injection ...	108	15	100
Later	81	13	100

(*b*) After administration of atropine. Injection of 100 c.c. saline solution, in 20″. Temp. 37·5° C.

Just before injection ...	138	11	120
At end of injection ...	160	8·5	130
Just after injection ...	150	13	120
Later	138	11	120

2. Dog: 10 kg. Temp. 35·5° C.

(*a*) Injection of 70 c.c. saline solution, in 20″. Temp. 35·5° C.

Just before injection ...	69	6	140
Just after injection ...	108	9	150
10″ after injection ...	93	7	140
Later	70	6	140

(*b*) After administration of atropine. Injection of 100 c.c. saline solution, in 25″. Temp. 35·5° C.

Just before injection ...	122	6	140
Just after injection ...	140	11·5	140
Later	122	8	140

The reflex effect after the administration of atropine is in large dogs beyond dispute, the slight effect found in cats and small dogs may perhaps be due to the sympathetic tone being not so easily affected by the rise of venous pressure or to its being too strong to show any further reflex increase.

From the experiments on frogs, rabbits, cats and dogs we can confirm the conclusion drawn by Bainbridge that the acceleration of the heart produced by the injection of fluid is reflex in origin and is due almost entirely to the diminution of vagus tone, to a small extent to increased accelerator tone. The latter we found usually difficult to obtain. The afferent fibres of the reflex probably only occur in the vagus stem. The negative effect found on frogs and rabbits is obviously due to absence of normal vagus tone.

Distribution of afferent fibres.

Method. Following the suggestion of Prof. Starling we used the method of distending different parts of the vascular system by means of a balloon. The experiments were made in two ways: (1) a metal tube with a small hole at one end covered with a rubber membrane, was passed down the v. jug. ext. into any desired part of the v. cava sup. or inf. (or sometimes in the right auricle). The other end of the tube was connected with a syringe by means of which air was pressed into the balloon to distend the vein. (2) The chest of the animal was opened and the right or left auricle was exposed. At the appendage a small hole was made through which a tube with a rubber balloon at its end was introduced into the auricular cavity. Sometimes a tube without a rubber balloon was inserted and Ringer's solution was injected into the auricle. Recording arrangements for the venous pressure and pulse-rate were the same as those used in those already described.

The right auricle. The distension of the right auricle causes definite acceleration of the heart usually accompanied by a rise of venous pressure without inducing necessarily any marked changes in the arterial pressure, provided that the distension lasts for some time (cp. the accompanying protocols). Short duration, *e.g.* one second, has no accelerating effect.

1. Dog: 9·7 kg. Temp. 36·5° C. Distension of the right auricle. Air, 8 c.c.

(*a*) Distension lasted 18″.

	Pulse rate	Venous pressure	Arterial pressure
Before distension ...	60	10	110
Just at end of distension	72	17	110
Later	60	10	110

(*b*) Distension lasted 23″.

Before distension ...	60	10	110
At end of distension	69	17	110
Later	60	10	110

2. Dog (Fig. 2): 7 kg. Temp. 36° C. Distension of right auricular appendage, duration 15″.

Before distension ...	72	9	105
At end of distension	90	9·5	105
Later	84	9	105
Later	72	9	105

The same effect was obtained when the sympathetic nerves had been cut.

The acceleration is reflex since it no longer occurs when all the cardiac nerves are cut.

Fig. 2. Showing the acceleration of heart-rate produced by the distension of right auricular appendage by means of balloon. Interval between two parts of tracing is 1′.

The veins. The effect of distension of the veins was next tried.

V. cava superior (close to the heart).

(*a*) Distension by means of balloon.

1. Dog: 7·5 kg. Temp. 36·5° C.

	Pulse rate	Venous pressure	Arterial pressure
Before distension ...	144	7	130
At end of distension	174	17	130
Later	144	7	130

2. Dog: 5·5 kg. Temp. 37·5° C.

	Pulse rate	Venous pressure	Arterial pressure
Before distension ...	90	5	110
At end of distension	138	10	110
Later	90	5	110

(*b*) Clamp.

3. Dog: 7 kg. Temp. 35·5° C.

	Pulse rate	Venous pressure	Arterial pressure
Before clamping ...	108	6	105
At end of clamping	123	12	100
Later	114	6	105
Later	108	6	105

No reflex acceleration was observed by clamping several parts of the v. cava superior or the v. jugularis, or by distension or stretching them.

It was not an easy task to obtain reliable results on the v. cava inferior, for its occlusion was usually accompanied by a great fall of arterial pressure. We met however with some cases where the arteria pressure remained relatively constant. The accelerating effect was obtained from the part close to the heart, but was not detected from the peripheral part.

1. Dog: 9 kg. Temp. 37° C. Distension of v. cava inf. (close to the heart) by means of balloon.

	Pulse rate	Venous pressure	Arterial pressure
Before distension ...	66	10	120
At end of distension	84	13	110
Later	66	10	120

2. Dog: 7·5 kg. Temp. 36·5° C.

Before distension ...	144	7·5	130
At end of distension	159	9	125
Later	144	7·5	130

Left auricle. The venous pressure was recorded in the jugular vein. Great decrease in the arterial pressure did not necessarily occur, when the auricle was distended, probably the full dilatation of the balloon was obstructed by the trabeculæ and a part of the auricular cavity still remained open for the passage of blood. It is also peculiar that a rise of venous pressure occurs in the jugular vein. This fact makes it difficult to interpret the results on the left auricle, for the rise of pressure in the right auricle causes, as we have seen, acceleration. We met however with several cases in which the accelerating effect was observed without an accompanying rise of venous pressure in the right auricle, and we conclude that impulses set up in the left auricle give rise to the acceleration. The following may be given as examples.

1. Dog: 7 kg. Temp. 37·5° C. Distension by means of balloon.

	Pulse rate	Venous pressure	Arterial pressure
Before distension ...	110	7	120
At end of distension	135	17	110
Later	110	7	120

2. Dog: 4·7 kg. Temp. 36° C. Injection of Ringer's solution 50 c.c. Temp. 36° C.

Before injection ...	99	7	120
At end of injection	117	10	120
Later	99	8	120

3. Dog: 9 kg. Temp. 36° C. Distension by means of balloon.

Before distension ...	96	9	125
At end of distension	114	9	125
Later	96	9	125

As in other cases the accelerating effect was not seen after dividing the vagi.

The adequate stimulus for the rise of venous pressure can only be the mechanical tension tangential to the wall. As a matter of fact, in the case of the left auricle, we met with reflex acceleration caused by the distension of its appendage without accompanying rise of venous pressure. Moreover we could produce the same effect by stretching the vena cava superior outwards.

SUMMARY.

1. The acceleration of the heart-rate produced by the injection of fluid causing a rise of venous pressure is, as Bainbridge concluded, reflex in origin and chiefly due to the diminution of vagus tone, partly to an increase of accelerator tone.

2. The greater the vagus tone, the more effective is the rise of venous pressure upon the acceleration of the heart in different species of animals or different individuals of the same animal.

3. In frogs and rabbits, where the vagus tone does not normally exist, no appreciable accelerating effect was found, the sympathetic nerves alone being, so far as our experiments go, not sufficient to produce this effect. The reflex acceleration due to increase of the accelerator tone was only clearly detected in some large dogs in which the heart-rate after administration of atropine remained relatively slow.

4. The distension of the auricles and of the great veins close to the heart, if it is of sufficiently long duration, produces acceleration of the heart-rate, which has the same cause as that brought about by the injection of fluid.

5. The reflex acceleration of the heart-rate may be produced by afferent impulses arising in both auricles, left as well as right, and in the great veins close to their openings in the auricle, but not in any peripheral veins; thus the afferent impulses probably travel up the vagi.

We are greatly indebted to Prof. Starling and Prof. Bayliss for their kind advice during our experiments.

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ON THE CORRELATION OF THE RATE OF HEART BEAT, BREATHING, BODILY MOVEMENT AND SENSORY STIMULI. BY WALTER M. COLEMAN.

MY first observations on this subject were made on animals in the Zoological Gardens, Regent's Park. The heart beats can be counted in various ways. It may be visible in the chest wall as in the squirrel, cheetah, leopard and seal; it may be shown by the vibrations of the whiskers; and sometimes, while the head is held up, the carotid pulse is visible.

The ratio of heart beat to the number of steps taken by the animals in quiet movement and to their ordinary quiet respiration was determined. It was found that usually the rate of heart beat was equal to, or an even multiple of, the rate of movement and breathing. The following are some of the observations made.

Animals	Duration of observations in seconds	Number of heart beats	Steps taken in walking	Ratio of breathing to heart beats
Civet ...	30	56 (seen on side)	56	1 to 4, 5, 4, 4, 4, 5
Cheetah ...	30	60 ,, ,,	60	1 to 5, 5, 6, 6, 6. 6, 4, 5
Leopard ...	30	80 (whiskers)	80	1 to 4, 4, 4, 4, 5

In 50 out of 300 observations the rate of steps and presumably of heart beat continually varied. But in nearly all these cases there was some obvious disturbing factor, such as the animals waiting to be fed, or a noise from the attendant. A bear walked at 10 steps in 6 seconds; teased by a boy, the steps were 10 in 6·2, 6·6, 5·8, 6·2, 6·6, 6·6, 6·6, 6·6, showing a recovery of steadiness in two minutes. The observations show, I think, that in animals when undisturbed there is a correlation between the discharge of nervous impulses causing movement, and the nervous discharge regulating the rate of the heart beat.

In order to test the matter further I have made observations in different ways on man.

(1) The subject was told to take his radial pulse with elbows held out, and, while taking the pulse, to walk with an easy swing at steady

pace paying more attention to his footsteps but continuing to observe his pulse. When the steps were faster than the pulse it was found that the pulse rate increased, and at rates within the limits of 90 to 120 a minute the pulse rate became that of the steps. If the test is repeated with the spine held stiff and the attention concentrated instead of easy, the pulse and steps fail to coincide.

(2) In the next test the subject's pulse was taken and he was then asked to walk in an indifferent mood. He was not told the object of the test. The rate of his steps was taken. 14 out of 24 subjects stepped at the rate taken for their pulse. As with several of the tests, it is best not to make this test twice on the same subject; for if he suspects that the influence of his heart on his footsteps is being tested, self-consciousness may become a source of error.

(3) The effect of attention to periodic sounds was also tried. The subject's pulse rate was taken for 15 seconds. The subject sat erect and poised and a metronome was placed before him and set beating at a rate 15–30 faster, or 10–15 slower, than his pulse. He was asked to give his undivided attention to the sounds. After half a dozen beats, his pulse was again taken for 15 seconds. It was found in nine cases out of ten that the pulse rate had become that of the metronome; thus in different observations the rate changed from 60 to 72, from 64 to 84, from 80 to 108, and from 56 to 48. The subject is not to be told that his pulse is expected to change its rate; and the clicking sound is not allowed beforehand lest his ears become tired. The attention lapsed or the mind wandered in some cases in less than half a minute. Those with power of sustained attention can keep the pulse in accord with the clicks for several minutes. With most subjects there was a perceptible swing of head to right and left. If tracings of the pulse changes are taken, the self conscious state induced becomes a source of error, especially if a sphygmograph is used with the uncomfortably high pressure necessary for air-transmission and the body held still to prevent risk of spoiling the record. The pocket Dudgeon sphygmograph used at its lightest pressure does not seem to interfere with the response; the records show the transition to a new rate to take place within 2 or 3 heart beats.

(4) Subjects singing with others were asked to pause in the song and test the pulse. It was found as a rule to keep time with the music.

(5) In a number of instances, while listening to a fluent or impressive speaker an auditor tested his own pulse and found a pulse-beat to fall with each accented syllable of the speaker's words.

(6) The rate of the heart may also be brought into time with move-

ment without the aid of impulses from the volitional centre by passively swinging the arm or leg of the subject at regular intervals.

That sudden changes in intra-thoracic pressure from sudden opening and closing of the glottis may be a factor in controlling the heart is indicated by the two following tests; which may also explain why many persons cannot take their own pulse without changing its rate:

The pulse of one person is taken by another and then by himself; if the rates differ he takes his own pulse again, this time only counting it mentally, keeping the throat relaxed and open. The pulse in most cases will now agree with the rate found by the first observer. The second test is to take the pulse in the relaxed way just described, and then to take it counting aloud or in a forcible whisper, calling each count a little ahead of the pulse beat. Its rate will thereby be quickened 10 or 15 p.c. Then it is taken with the count slightly dragging after the beats; this retards the rate 10 or 15 p.c. Since the counting is continually accelerated or retarded the pulse is not allowed to catch up with the counting.

Observation of the bare abdominal wall showed that for each act of attention, whether sensory or motor, there occurred a slight twitch or pause in the respiratory movement of the wall. Thus the muscular wall of the body, acting with the diaphragm, and perhaps aided by the "stroke of the glottis," probably gave mechanical stimuli to the heart, bringing it to the new rate. If the subject sat in a collapsed posture, instead of erect and poised, the pulse-rate was not controlled by attention to periodic stimuli. Since the pericardium is bound down to the diaphragm, each respiratory movement acts directly upon it.

Besides the sudden dynamic changes in pressure upon the heart just mentioned, its rate is probably influenced by the mechanical effects of the footsteps and other intermittent movements; for it is found that if all interruptions and jarring are avoided, as in using a bicycle ergometer where the rhythmic changes in exertion are very gradual, the movements fail to impart their rate to the heart. If by cutting out sudden periodic changes the effect ceases it is probable that they are necessary to the effect.

I have several times pointed out that stiffness interferes with the response of the heart to rhythm; this effect is probably likewise due to the stiffness preventing intermittence (in exertion and relaxation). The stiffness may be occasioned by self-consciousness, by emotion, or by the attachment to the body of delicate apparatus, the use of which requires immobility. The heart seems to take up a new rate best when the body and head are free to swing from side to side. For anatomical reasons the pendulum effect of a right and left swing is more regular than the effect of a swing back and forth.

The experiments seem to show that voluntary attention involves muscular activity. Yet it appears that mere voluntary attention alone, which does not also include intermittent, periodic action of the respiratory muscles, will not bring the heart to the rate of the stimulus. However, the stimulus may disturb the heart and change its rate, for doubtless, in all voluntary attention, impulses irradiate directly to the heart by way of the vagus. Though there is doubtless associated activity of this mechanism, the response of the heart to external periodic stimuli appears to be not so much direct as mediated through the rhythmic action of the glottis, abdominal wall and swings of limb or trunk, though the last may be so slight as to pass unperceived.

These bodily responses are prevented by rigidity; and this in turn has its source in strain from unsymmetrical posture, or in pain or discomfort or in the immobility, for instance, necessary during observations with delicate apparatus where any motion of the subject would spoil the record. Stiffness may also arise in the self-consciousness of tests made before spectators. Any emotional, asphyxial or apnœic condition which necessitates irregular breathing will obviously interfere with the regular action of glottis, body wall and the rhythmic swing.

In man as in the lower animals, as has already been shown, irregular rhythm of body and probably also of heart forms a part of emotional reaction. This tends to prevent the taking up of regular rhythm; for I find that a heart that is already beating at a regular rate will fall in with periodic stimuli of a new rate when an irregularly beating heart remains unaffected.

Metabolic demands upon the breathing cannot, of course, set the heart at a definite rate, but those demands must not place a hindrance in the way of the respiratory response to periodic stimuli. For instance in reducing the pulse rate by attention to the slow beats of a metronome the heart did not slow its rate unless the breathing was relaxed and the sounds counted in a drone or drawl in a half indifferent mood. Experimental evidence of this is found if the test of the heart for periodic response is combined with the writer's test (this *Journal*, **53**, p. 361) for ventilation of the blood: it will be found that the heart takes new rhythms best when ventilation is balanced, this being known by the succession of paired and unpaired images. (For this test the subject must be normal, not one who gets a fixed image, nor one—usually over 60 years old—who, because of conjugate deviations, gets no image.)

The problem has been, not to study the well-known responsiveness of the heart to almost all stimuli, but to find under what conditions, if any, the heart will keep in time with perfectly periodic stimuli.

SUMMARY.

1. Accord of rates is usual between heart, footsteps and breathing.

2. The heart will take up the rate of periodic movements or sensory stimuli within limits (about 15 p.c. below or 30 p.c. above its rate) if the body is free of stiffness, in erect, easy poise, and the attention steady but flexible.

3. The readjustment begins at once and is completed in about three heart beats.

4. Emotion, strong exertion, or unusual metabolic demands may prevent the accord of rates.

I am greatly indebted to Professor Langley for assistance in this work.

STUDIES ON MUSCULAR CONTRACTION. I. The influence of temperature on the mechanical performance of skeletal and heart muscle. By YASUKAZU DOI, M.D. (Japan).

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It is well known that the duration of a muscular contraction is shorter at a higher temperature than at a lower. The influence of temperature on the mechanical performance of a muscle when it contracts has, however, still remained obscure. Gad and Hymans(1) studied this problem and found that between 0° C. and 19° C. the tension developed in a frog's muscle during its isometric contraction, or the work done on its isotonic contraction, is larger at a lower temperature than at a higher. Bernstein(2) got the result that the temperature coefficient of mechanical performance of the muscle is sometimes positive and sometimes negative, *i.e.* the tension developed, or work done, by a muscle is sometimes larger and sometimes smaller at a higher temperature than at a lower. He explained the result as follows: When a muscle is excited by a stimulus the muscle substance undergoes a chemical reaction, liberating an amount of chemical energy from its store (total energy in terms of thermodynamics). This chemical reaction being an exothermic one, a portion of the total energy set free may be converted into mechanical work (free energy in terms of thermodynamics) when the muscle is under a suitable condition, while the rest of the total energy is liberated in the form of heat. Assuming that the temperature coefficient of the total energy change in the contractile process is positive, viz. the higher the temperature, the more total energy is set free on contraction, it is concluded from the results above mentioned, that the process of conversion of the total energy into mechanical work has a negative temperature coefficient. The temperature coefficient observed in an experiment is the sum of these two temperature coefficients of opposite signs. Either of them may overcome the other, and the observed temperature coefficient will have the sign of the more powerful one.

It is not correct, however, to assume that the temperature coefficient of the total energy change is positive. It seems that

Bernstein has confused the total energy set free in a chemical reaction with the reaction velocity. The temperature coefficient of the velocity of a chemical reaction is always positive, but the total energy set free in the reaction is independent of its velocity. The temperature coefficient of the former depends upon the nature of the reaction. In the contractile process of the muscle it is impossible to determine it, for the nature of the reaction is quite unknown. The only evidence in support of his assumption is given by the experiment of **Fick**(3), who stated that the heat production on muscular contraction is larger at a higher temperature than at a lower. According to A. V. **Hill**(4), however, **Fick's** experiments on heat production of muscle cannot be accepted without further test. So long as the temperature coefficient of the total energy set free remains unknown, that of the conversion of the total energy into mechanical work cannot be decided to be negative, as **Bernstein** ventured to affirm, but is left quite undetermined. No matter what the temperature coefficient of the total energy change may be, the temperature coefficient of the mechanical performance observed in the experiment is that of free energy developed.

It has been pointed out by **Hill** that the power of a muscle to do work in contraction can be estimated by the tension developed in the muscle when it contracts isometrically. The work done by a muscle on its isotonic contraction is also a function of its power of doing work, but is complicated by other factors, such as the weight of load and the change of length during the process of contraction, so that the power of doing work by the muscle cannot be estimated by this method.

The figures of **Bernstein's** experiments show that the tension developed in the isometric contraction caused by a break induction shock (which is probably the maximal stimulus) has in most cases a negative temperature coefficient.

On the other hand the tension developed in a muscle on its isometric contraction varies according to its initial length, other conditions being equal. The experiments of **Blix**(5) and of **Evans** and **Hill**(6) show that the tension developed increases with increasing length of muscle up to a certain optimal length. Beyond this limit the tension developed decreases with increasing length of the muscle. It was proved by **Patterson, Piper** and **Starling**(7) and **Kozawa**(8) that the same law applies to the mechanical performance of the heart. Hence the initial length of the muscle may affect the temperature coefficient of the tension developed.

Thus the influence of the temperature on the mechanical performance of the muscle must be studied taking the length of the muscle as a variable factor. At the suggestion of Prof. Starling the experiments, of which the results are described below, were undertaken on the skeletal and heart muscle of the frog in order to obtain some direct information on this point.

Methods. The muscle employed is the gastrocnemius of *Rana esculenta*. The proximal end of the excised muscle is attached by a hook to the tension lever designed by Blix(9), which records the initial tension of the muscle at rest and the total tension sustained by it on contraction. The muscle is suspended in a glass vessel of about 30 c.c. containing Ringer's solution. To the tendo Achillis of the muscle is hooked a steel needle, which passes through a hole in the bottom of the vessel which is plugged with vaseline. The other end of the needle can be held tight and lowered at will by means of an adjusting screw. The needle has a horizontal branch outside the vessel which records the length of the muscle, so that the tension developed and the initial length can be recorded at the same time on a stationary drum. The needle passes through a tube fixed vertically, of about 5 cm., the calibre of which just fits the needle. Thus the needle is compelled to move strictly vertically, and no error can affect the record of the length of the muscle. The needle with its horizontal branch weighs about 2 grams. The actual load on the muscle, however, is not the whole weight of the needle, when it is not drawn downwards, but only a small portion of its weight, the greater part being supported by the vaseline plugging the hole through which the needle passes. The length of the muscle thus loaded with a minimal weight is taken arbitrarily as its unloaded length, as it is a matter of considerable difficulty to record the length of a perfectly unloaded muscle. The spring of the tension lever is a strong one, so that the displacement of the lever at the point of attachment of the muscle is negligible compared with the length of the muscle.

As stimuli, maximal opening shocks are employed, applied between the two metal hooks described above, which attach the muscle to the tension lever and the adjusting screw respectively. The leakage of the stimulating current into the solution immersing the muscle is prevented as far as possible by coating with vaseline the hook attached to the muscle, which comes in contact with the solution. Care is taken on each record to test that the tension developed remains the same when a stronger stimulus is applied, so that the contraction is really a maximal one. The temperature of the Ringer's solution can be changed gradually and kept constant at any desired degree by pouring slightly warmer or colder solution into the vessel and draining out the excess of solution by means of a siphon.

In my experiments, the tensions developed are recorded at 5° C. and 15° C. respectively. These two degrees are chosen, since both are within the physiological range of temperature for the frog. The tension developed is estimated first at one degree, next at the other, and then again at the first degree and it is shown that the tensions are the same at the first and the third estimations, so that fatigue and exhaustion do not affect the result. The tensions developed at the two degrees of temperature are measured, first at the assumed unloaded length of the muscle, and then at different lengths produced by extension.

The methods and procedure of the experiments made on the heart of the frog are in principle similar to those on the skeletal muscle. In this case, however, the intra-ventricular pressure, measured isometrically, of the heart contracting spontaneously, is recorded on a drum by means of a Hürthle's manometer, instead of recording the tension developed in the fibres themselves, and the filling of the heart is taken as a variable factor in place of the length of the muscle (cp. Patterson, Piper and Starling(7)). The arrangement of the experiment is similar to that described by Kozawa; and the details need not be repeated. The desired temperature (5° C. or 15° C.) is applied by means of Ringer's solution, in which the isolated heart is immersed. The Ringer's solution which flows into the heart is also kept at the same temperature, by passing through a silver spiral tube which is put in the solution immersing the heart. The temperature of the solution surrounding the heart and the spiral tube is adjusted in the same way as in the experiment on the skeletal muscle.

The pressure is recorded first at 5° C., then at 15° C., and again at 5° C., and those experiments in which the influence of fatigue or exhaustion can be seen are rejected. This precaution is particularly necessary in the case of the heart, for it often happens that the pressure diminishes after repeated contractions, especially when the heart is dilated to a considerable extent.

Results and discussion.

In discussing the results of the experiments, I have considered only the tension developed on contraction, viz. the difference between the total tension which the contracted muscle developes and the initial tension to which the resting muscle is subjected, but not the total tension developed, for the former is, according to Evans and Hill, the one which corresponds to the energy output of the muscle on contraction.

On the skeletal muscle four experiments were made which gave similar results. A typical one is given in Table I with corresponding Fig. 1, in which the abscissæ represent the length of the muscle and the ordinates, the tension developed. The general type of the relation between the tension developed and the length of the muscle is the same as seen in the experiments of Blix, and of Evans and Hill. The tension developed increases with increasing length of the muscle up to a certain optimal length. When the muscle is stretched beyond this optimum limit, the tension developed decreases with increasing length. The optimal

TABLE I.

Length of muscle mm.	Initial tension grams		Total tension sustained on contraction grams		Absolute tension developed on contraction grams	
	5° C.	15° C.	5° C.	15° C.	5° C.	15° C.
23·7	0	0	125	80	125	80
24·8	5	5	175	125	170	120
25·8	20	20	205	150	185	130
26·9	40	40	215	190	175	150
27·8	80	80	225	215	145	135
28·7	120	120	225	225	105	105
29·8	170	170	230	240	60	70
31·2	220	220	240	260	20	40

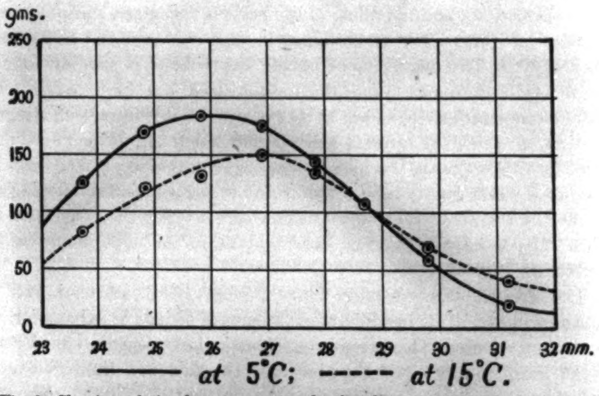

Fig. 1. Showing relation between tension developed on contraction and length of muscle at different temperatures.

length of the muscle is shorter and the tension developed at this length is higher, at a lower temperature than at a higher. In the example given, the optimal length is about 26 mm. with a tension of 185 grams at 5° C., while at 15° C. it is 27 mm. with a tension of 150 grams. The increase of the tension developed with increasing length up to its optimum is more rapid, and the decrease of the tension, when the muscle is stretched beyond the optimum limit, is also more rapid, at the lower temperature than at the higher, so that the descending branch of the tension curve of the lower temperature cuts that of the higher. In other words, the tension developed is higher at the lower temperature than at the higher, *i.e.* the temperature coefficient is negative, when the length of the muscle does not exceed its optimum. Beyond this optimal length the difference of the two tensions decreases with increasing length of the muscle, until at last the tension at the lower temperature becomes lower than that at the higher, so that the temperature coefficient is positive. This can be explained by the fact that the optimal length is shorter at the lower temperature than at the higher. When muscle is stretched beyond its optimal length it is no more under the physiological condition, but is in an abnormal state, so that the muscle substance no longer exerts its utmost performance—in other words the functional capacity of the active surface of the muscle fibre is suffering from some injury caused by extreme extension. The intensity of this injury is as a matter of fact larger with increasing deviation of the length of the muscle fibre from the optimum. When a muscle fibre is stretched to a certain abnormal length, the deviation of its length from the optimum is greater, and accordingly the injurious action is larger at the lower temperature than at the higher, for the optimal length is shorter in the former case than in the latter. Hence the mechanical performance of a muscle under extreme extension at the higher temperature may exceed that at the lower.

On the heart similar results were obtained in all 11 experiments made. Table II with corresponding Fig. 2 is a typical example of them, in which the abscissæ represent the filling of the heart, the ordinates, the intraventricular pressure. This example shows that the relation between the two pressure curves at a higher and a lower temperature respectively is just the same as in the case of the skeletal muscle, *i.e.* the heart muscle is subjected to the same law as that which governs the skeletal muscle as to the influence of temperature on its power of mechanical performance. In the heart the difference of the optimal fillings at each temperature is especially remarkable. In the example given the optimal filling at 5° C.

is ·03 c.c., while it is ·06 c.c. at 15° C. It is seen in this example that a slight pressure is set up on contraction even when the filling is zero. Theoretically this ought not to be the case. The reason is that the heart at its supposed zero filling still contains a minimal amount of solution. It is difficult to connect the perfectly empty heart with a Hürthle's manometer.

Table II.

Filling c.c.	Initial tension mm. Hg.		Total tension sustained on contraction mm. Hg.		Absolute tension developed on contraction mm. Hg.	
	5° C.	15° C.	5° C.	15° C.	5° C.	15° C.
0	0	0	3·8	3·2	3·8	3·2
·01	·4	·4	34·3	17·1	33·9	16·7
·02	·5	·5	43·4	27·9	42·9	27·4
·03	1·2	1·0	50·0	30·7	48·8	29·7
·04	1·2	1·2	47·6	39·1	46·4	37·9
·05	2·0	1·3	46·8	40·0	44·8	38·7
·06	2·0	2·0	43·7	43·3	41·7	41·3
·07	3·1	3·0	40·7	41·7	38·5	38·7
·08	6·2	5·7	40·0	41·7	33·8	36·0

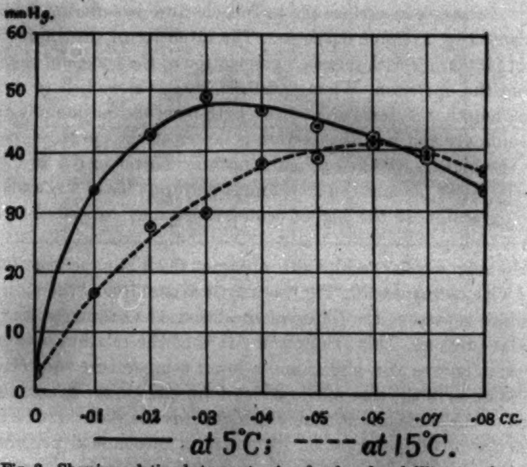

Fig. 2. Showing relation between tension developed and filling in a heart contracting isometrically at different temperatures.

SUMMARY.

The statement is confirmed that the mechanical performance of muscle increases with increased length up to a certain optimal length and decreases when the length exceeds this.

So long as the muscle is under physiological conditions with respect to its length, viz. if it is within the limit of the optimum, the power of mechanical performance is larger at a lower temperature than at a higher, so that the temperature coefficient of mechanical response is negative.

This optimal length of the muscle is shorter at a lower temperature than at a higher.

Under extreme conditions of overstretching the mechanical performance of the muscle at a lower temperature may be smaller than that at a higher. This can be explained by the change of the optimal length with respect to the temperature.

The above statements are found to apply to skeletal muscle as well as heart muscle.

In conclusion I wish to express my thanks to Prof. Starling for his suggestions and advice during the course of this work.

ADDENDUM.

Mr A. V. Hill suggested to me to repeat the experiment (1) with a tension lever much stronger than that of Blix, in order to record the tension set up by a muscle kept as completely isometric as possible, and (2) on sartorius muscle, for it has a uniform cross-section and straight fibres. The experiments were made in the Cambridge Physiological Laboratory and Mr Hill kindly allowed me to use the optical tension lever described by him in his recent paper (10). I am also indebted to him and to Mr Hartree for valuable advice in performing the experiments.

Hill's optical tension lever has a very strong spring, so that, when its points for attaching the muscle is loaded with 100 grams weight, this point shows a displacement of only ·03 mm., while giving a record of 30 mm. at a distance of about three metres. Its period of vibration is of the order of 10^{-3} sec. I performed four experiments on the double sartorius preparation with this lever, the other arrangements being the same as before, and obtained results in complete agreement with those previously found. One of them is given here as an example. In this experiment, the length of the resting muscle is 20 mm., and that of the freely contracted muscle without load is 17 mm. The extension of the muscle is measured starting from the latter length, so that the tension is zero at zero extension.

TABLE III.

Extension of muscle mm.	Initial tension grams		Total tension sustained on contraction grams		Absolute tension developed on contraction grams	
	5° C.	15° C.	5° C.	15° C.	5° C.	15° C.
5·0	0	0	5	3	5	3
10·0	1	1	23	12	22	11
12·0	3	3	27	17	24	14
14·0	5	5	32	22	27	17
15·5	10	10	36	30	26	20
17·0	15	15	35	35	20	20
18·5	32	32	45	45	13	13
20·0	57	57	64	65	7	8
22·0	77	77	80	82	3	5

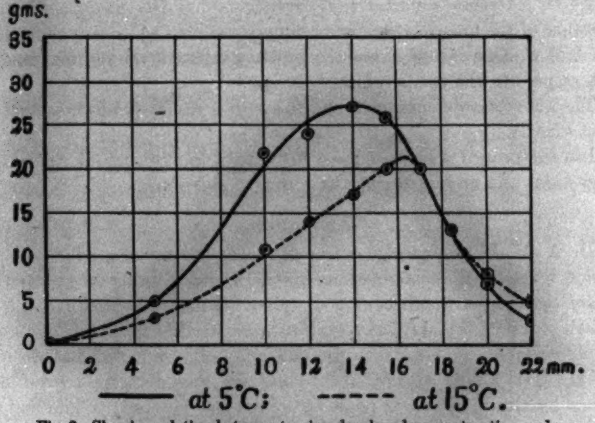

Fig. 3. Showing relation between tension developed on contraction and extension of muscle at different temperatures.

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ON THE EXISTENCE OF ANTIDROMIC FIBRES IN THE FROG AND THEIR INFLUENCE ON THE CAPILLARIES. By YASUKAZU DOI, M.D. (Japan).

(*From the Institute of Physiology, University College, London.*)

Antidromic fibres in the frog.

THE existence of vasodilator fibres in the posterior roots of the spinal nerves of mammals has already been conclusively shown by Bayliss(1, 2). Oinuma(3), however, as a result of experiments, in which he stimulated the roots electrically, denied their existence in the posterior roots of the frog, though he found vasodilatation in the frog when the sciatic was stimulated some days after section of the nerve—an effect which in the mammal is attributed by Bayliss to antidromic action. It appears unlikely that the frog has no antidromic fibres in the posterior roots, when we reflect upon the fact that the distribution of vasoconstrictor fibres in the frog—according to Langley(4)—is similar to that in mammals. And in view of this the problem has been subjected to a re-examination[1].

Methods. I have adopted two methods. The first consists in measuring the diameter of blood vessels in the web of an anæsthetised or decerebrated and curarised frog under a microscope, with the aid of an ocular micrometer. The magnification employed was such, that one division of the ocular corresponded to 4 μ.

The second method was plethysmography of the hind leg of a decerebrated and curarised frog. A glass plethysmograph was used (Fig. 1), the junction with the leg being made tight by means of vaseline. The plethysmograph was filled up with water and connected with a capillary tube by means of rubber tubing. The change in the height of the water surface in the capillary tube was read. A change of 1 mm. corresponded to 0·88 c.mm. When a frog's leg is correctly and securely fitted in the plethysmograph the water surface in the capillary shows pulsation corresponding to each heart beat.

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[1] While this paper was in print my attention was called to a paper by Pearce (*Ztschr. f. Biol.* 62, p. 243. 1913) in which he describes vasodilatation on stimulating the posterior roots of the frog, using Trendelenburg's method.

As stimuli for the posterior roots pinching with a forceps was employed instead of electrical stimuli, in view of the fact that mechanical stimulation has been found to be more effective on antidromic fibres, than electrical stimulation. In all cases the stimulus was a single pinch of the nerve. Mechanical stimulation has also the advantage that there is no question of escape of current to the anterior roots. Ten experiments were made with similar results. Excitation of the peripheral end of the 8th and 9th divided posterior roots, and sometimes of the 7th root also[1], caused an increase in the volume of the hind leg of the corresponding side. This increase in volume does not begin until about 10–20 seconds after the stimulus is applied, and its maximal point is reached 1–2·5 minutes after its commencement, then after remaining from one to three minutes at its maximum, the volume begins to decrease to the initial value which is reached after a period of 10 to 25 minutes. In order to obtain this result it is necessary to wait, 20 to 30 minutes after the root is cut, before applying a stimulus, since cutting the root itself acts as a stimulus, and causes a volume change like that stated above.

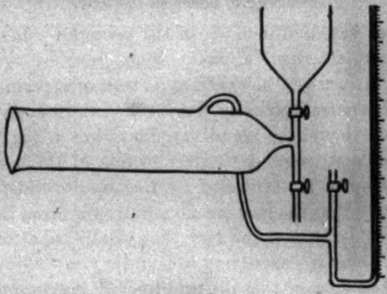

Fig. 1.

These results are illustrated by the example given in Table I and Fig. 2, which shows, too, that cutting the anterior roots causes a diminution in volume in a similar period of time as the dilatation caused by the posterior roots. This diminution is undoubtedly due to the stimulation of vasoconstrictor fibres contained in the anterior roots.

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[1] According to some anatomists the ten roots of the spinal nerves of frogs are counted two to eleven, the degenerated root being counted as one (see Ecker-Gaupp). Nevertheless, here we shall adopt the physiologists' notation by counting one to ten.

TABLE I. Change in volume of a leg caused by cutting and stimulating the roots (Fig. 2). Frog 37 grams. Room temperature 14° C.

Root cut	Time (minutes)	Change in vol. (c.mm.)	Root stimulated	Time (minutes)	Change in vol. (c.mm.)
	0	0	post. 9th	63	0
post. 9th	3	0		64	+6·2
	4	+6·2		66	+6·2
	5	+6·2		78	0
	15	0	post. 8th	81	0
post. 8th	20	0		82	+3·5
	21	+3·5		84	+3·5
	22	+3·5		94	0
	30	−2·6	post. 7th	97	0
post. 7th	33	−2·6		99	0
	34	−2·6		105	0
	39	−2·6			
anterior roots (6th, 7th, 8th, 9th and 10th) cut					
	40	−4·4			
	41	−4·4			
	60	0			

In this and the following tables the change from zero is given, thus in the foregoing table the volume of the limb was the same at the 5th minute as at the 4th.

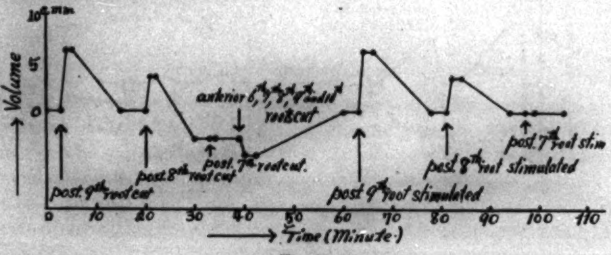

Fig. 2.

The increase in volume caused by stimulating the 9th posterior root, in the majority of cases, slightly exceeds that caused by the 8th root, which is usually thinner than the former. A slight increase in volume is sometimes given by stimulation of the 7th root, sometimes, however, no effect is observed. It depends on how much the thigh is put in the plethysmograph. During the increase in volume of a leg caused by stimulating posterior roots, no change of blood-pressure occurs, so that this increase in volume is not due to any change in the blood-pressure. The increase in volume is due mainly to dilatation of the skin vessels, as was shown by Bayliss in the case of mammals. If, after recording the increase in volume of the leg, caused by stimulating the posterior root, the same leg is skinned, and the bared leg is again fixed in the plethysmograph, then, the root being stimulated, the increase in volume is found to be very minute. This is shown in Table II and Fig. 3.

TABLE II. Change in volume of a leg caused by stimulating the posterior roots before and after the skin is stripped off (Fig. 3). Frog 42 grams. Room temperature 15° C.

Anterior roots 6th–10th and posterior roots 7th–10th were cut previously.

Root stimulated	Time (minutes)	Increase in vol. (c.mm.)	Root stimulated	Time (minutes)	Increase in vol. (c.mm.)
	0	0	The leg is skinned.		
post. 9th	3	0		0	0
	4	6·2	post. 9th	3	0
	6	6·2		4	0·9
	17	0		5	0·9
post. 8th	20	0		10	0
	21	4·4	post. 8th	15	0
	23	4·4		16	0·5
	33	0		17	0·5
post. 7th	35	0		20	0
	36	0·9	post. 7th	25	0
	37	0·9		26	0
	43	0		30	0

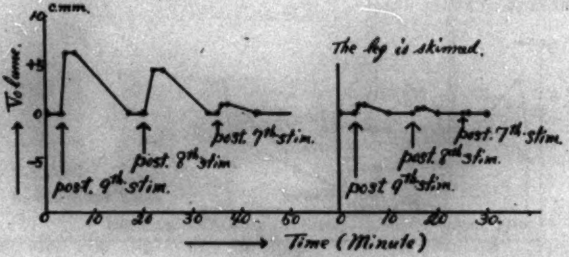

Fig. 3.

Similar conclusions to those arrived at above are obtained by the other method, namely by microscopic observation. A remarkable dilatation is shown by the vessels of the web of the leg on stimulating the corresponding posterior roots. It is not only the arterioles, which show dilatation, but also the capillaries and even the venules dilate remarkably. Dilatation of the small vessels is accompanied by a marked increase in the amount of blood contained in them, a greater or less retardation of the blood flow through them, and sometimes a marked pulsation, especially in the capillaries. Table III is an example of this kind of experiment; ten other observations gave like results.

The duration of the dilatation, after stimulation of the root, is quite similar to that described above in the case of the plethysmograph, so that the same precaution of waiting 20 to 30 minutes after cutting the root, before applying a stimulus, is necessary.

TABLE III. Dilatation of vessels caused by stimulating the posterior roots. Frog 30 grams. Room temperature 15·2° C.

2nd web

	Diameter (micrometer division)	Diameter (μ)	Change in diameter (μ)
	capillary		
normal	3·3	13·2	
post. 8th stimul.	3·9	15·6	+2·4
post. 9th stimul.	3·3	13·2	0
	arteriole		
normal	7·1	28·4	
post. 8th stimul.	8·0	32·0	+3·6
post. 9th stimul.	7·1	28·4	0
	venule		
normal	4·7	18·8	
post. 8th stimul.	4·9	19·6	+·8
post. 9th stimul.	4·7	18·8	0

3rd web

	Diameter (micrometer division)	Diameter (μ)	Change in diameter (μ)
	capillary		
normal	2·7	10·8	
post. 8th stimul.	2·7	10·8	0
post. 9th stimul.	3·2	12·8	+2·0
	capillary		
normal	3·1	12·4	
post. 8th stimul.	3·4	13·6	+1·2
post. 9th stimul.	3·1	12·4	0
	arteriole		
normal	6·3	25·2	
post. 8th stimul.	6·7	26·8	+1·6
post. 9th stimul.	6·3	25·2	0
	venule		
normal	9·0	36·0	
post. 8th stimul.	9·1	36·4	+ ·4
post. 9th stimul.	9·0	36·0	0

The influence of antidromic action on the capillaries.

In the preceding section it has been shown that both arterioles and capillaries dilate simultaneously on stimulating the posterior roots. There can be scarcely any doubt that the arterioles are subjected to the control of antidromic fibres. In regard to capillaries, however, it can not yet be concluded from the results obtained above, that they are dilated by the direct action of vasodilator fibres. When arterioles are dilated, the pressure is raised in the capillaries, which may cause them to dilate, so that this dilatation may be only a secondary effect following the dilatation of the arterioles. Krogh(5) observed a dilatation of the capillaries in the tongue of a frog, on the application of a mechanical stimulus to the surface, and ascribed it to the action of a local axon reflex conveyed along sensory fibres. So far as I know, however, no definite evidence has yet been furnished of the direct action of antidromic fibres upon capillaries. This action I have investigated making use of the effect which Dale and Richards(6) have shown to be produced by histamine and acetylcholine.

Histamine. Dale and Richards showed that in the dog and cat histamine causes capillary dilatation, while it causes the arterioles to contract slightly, and in addition that it has a direct action upon the endothelial cells of the capillary, and not on its nervous elements. In studying the action of histamine on the blood vessels of the frog, I prepared solutions of various strengths of histamine from commercial tabloids of ergamine acid phosphate, of which 3 mgms. are equal to 1 mgm. histamine. ·05 c.c. solution was injected into the abdominal vein, an injection of the same quantity of saline solution having previously been proved to have no influence on either the blood-pressure or the volume of the leg. It was first ascertained, that the injection of ·0001 mgm. histamine into a frog of about 40 grams, caused an increase in volume of the leg, accompanied by a slight fall in the blood-pressure almost synchronous with the increase in volume, while ·001 mgm. histamine caused a more definite increase in volume together with a rise of blood-pressure. The former dose was therefore adopted throughout the experiments. Table IV and Fig. 4 is the protocol of one of these experiments.

Under the microscope it is seen that injection of histamine induces a marked dilatation of capillaries, although arterioles and venules remain unaffected or the arterioles are even to a slight extent contracted. The capillary dilatation begins about 10 to 30 seconds after the injection, increases for two to three minutes, when it arrives at its maximal dilatation, in which state it remains from two to four minutes, and then from this point begins to reproduce the initial state, which is only attained after a lapse of 10 to 25 minutes. It may be mentioned that during the first few seconds a state of anæmia is often seen which however soon disappears.

With the aid of this action of histamine the direct vasodilator action of the antidromic fibres on the arterioles can be proved. While histamine is exerting its dilator action on the capillaries, a stimulus is applied to a posterior root, which has already been isolated from the cord some time previously. A marked dilatation of the arterioles and a more or less increased dilatation of capillaries and venules results and can be seen under the microscope. Table V, one of these experiments, indicates these results.

Instead of examining the web, volume changes of the hind limb can be studied under similar conditions. The combined magnitude of the increase in volume, caused by the injection of histamine and stimulation of the 8th and 9th posterior roots, is nearly the same as that caused by the simple stimulation of the roots, before injection of histamine, so that the degree of dilatation of vessels attained by both experiments is practically the same. Table VI and Fig. 5 is an instance of this kind of experiment.

TABLE IV. Change in volume of a leg and in blood-pressure caused by histamine injection (Fig. 4).

Frog 38 grams. Room temperature 14·8° C.

Time (minutes)	Increase in volume (c.mm.)	Blood-pressure (cm. Hg.)	Change in blood-pressure (cm. Hg.)
0	0	3·8	0
3*	0	3·8	0
4	4·4	3·3	–·5
5	6·2	3·3	–·5
6	7·0	3·3	–·5
10	7·0	3·4	–·4
12	5·3	3·4	–·4
15	4·4	3·5	–·3
21	2·6	3·5	–·3
25	1·8	3·65	–·15
30	·9	3·7	–·1
38	0	3·8	0
40	0	3·8	0

* Histamine injection.

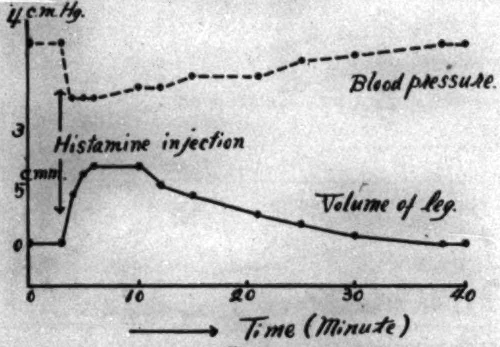

Fig. 4.

TABLE V. Dilatation of vessels caused by histamine injection and stimulation of the posterior roots. Frog 34 grams. Room temperature 14·7° C. The anterior and posterior roots cut previously.

	Diameter (micrometer division)	Diameter (μ)	Change in diameter (μ)
		capillary	
normal	2·6	10·4	
histamine injection	2·6	11·6	+1·2
posterior roots stimul.	3·0	12·0	+ ·4
		arteriole	
normal	5·7	22·8	
histamine injection	5·5	22·0	– ·8
posterior roots stimul.	6·3	25·2	+3·2
		venule	
normal	4·6	18·4	
histamine injection	4·6	18·4	0
posterior roots stimul.	4·7	18·8	+ ·4

In this example, it can be seen that the increase in volume caused by histamine, which is due solely to dilatation of capillaries, is 5·3 c.mm., while the further increase caused by the stimulation of posterior roots (in which increase is included that due to the dilatation of capillaries and venules) is only 1·7 c.mm. Three other experiments give respectively 7·0 c.mm., 4·4 c.mm., and 5·3 c.mm. for the former increase, and 2·7 c.mm., 2·2 c.mm., and 1·7 c.mm. for the latter. If we may be permitted to deduce a quantitative conclusion from these four experiments, it may be said that, more than two-thirds of the aggregate volume increase results from the dilatation of capillaries.

Table VI. Change in volume of a leg caused by histamine injection and stimulation of the posterior roots (Fig. 5). Frog 36 grams. Room temperature 14·5° C.

	Time (minutes)	Increase in vol. (c.mm.)		Time (minutes)	Increase in vol. (c.mm.)
	0	0	post. 8th and 9th stim.	50	0
histamine injection	3	0		52	6·2
	4	2·6		55	6·2
	5	4·4		59	4·4
	6	5·3		68	3·5
post. 8th and 9th stim.	8	5·3		74	2·6
	10	7·0		79	1·8
	12	7·0		84	·9
	13	6·2		90	0
	16	4·4		95	0
	21	2·7			
	28	1·8			
	40	·9			
	46	0			

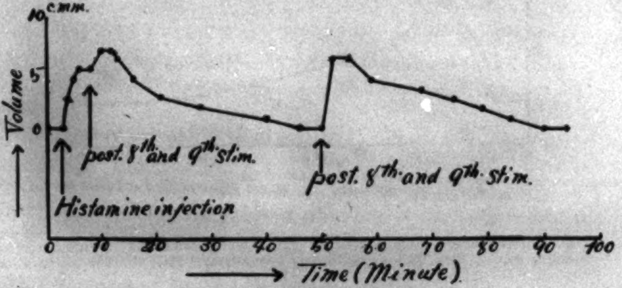

Fig. 5.

Acetyl-choline. Hunt(7, 8), and Dale and Richards proved independently, that the injection of a minute amount of acetyl-choline causes in various mammals a dilatation of blood vessels, accompanied by a fall in blood-pressure. They concluded that this action of the drug is peripheral, and not nervous. The same action I found to be produced in the frog. The procedure of the injection was similar to that described above in the case of histamine. An injection of ·0000025 mgms. acetyl-choline in a frog weighing about 40 grams causes an increase in volume of the hind leg, accompanied by a fall in the blood-pressure, almost parallel to the change in volume. A slight and temporary decrease in volume is often seen preceding the increase. This is shown in Table VII and Fig. 6, which is one of five experiments made.

TABLE VII. Change in volume of a leg and in blood-pressure caused by acetyl-choline injection (Fig. 6). Frog 31 grams. Room temperature 14·7° C.

Time (minutes)	Increase in vol. (c.mm.)	Blood-pressure (cm. Hg.)	Change in bl.-pr. (cm. Hg.)
0	0	2·8	0
3*	0	2·8	0
4	1·8	2·85	+·05
6	3·5	2·5	–·3
8	3·5	2·5	–·3
12	3·5	2·55	–·25
19	2·6	2·6	–·2
22	1·8	2·7	–·1
26	·9	2·8	0
31	0	2·8	0
39	0	2·8	0

* Acetyl-choline injection.

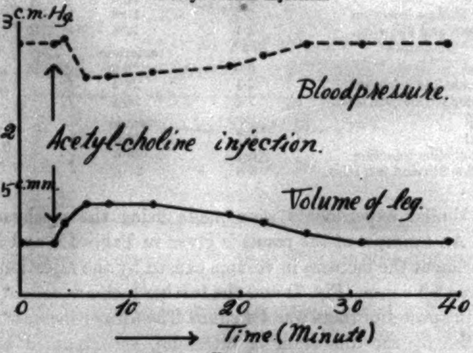

Fig. 6.

Microscopic observations indicate that, in the first few seconds following the injection, a strong constriction of vessels occurs in certain districts of the web (not all over the web), which however soon disappears, in the same manner as mentioned by **Hunt**, and **Dale** and **Richards** in mammals, then, the arterioles show a marked dilatation, accompanied in most cases by a slight dilatation of the capillaries, while the venules remain unaffected. The duration of the dilatation of the arterioles is almost identical with that caused by histamine.

With the aid of this action of acetyl-choline on the arterioles the direct dilator action of the antidromic fibres on the capillaries can be proved. After a maximal dilatation of arterioles has been attained by means of acetyl-choline, the peripheral end of the dissected posterior roots are stimulated. This causes a remarkable dilatation of the capillaries and sometimes a slight dilatation of venules, while the arterioles remain in their dilated state unchanged. This is clearly seen in Table VIII, which is one of three experiments done.

TABLE VIII. Dilatation of vessels caused by acetyl-choline injection and stimulation of the posterior roots. Frog 37 grams. Room temperature 14·4° C. The anterior and posterior roots cut previously.

	Diameter (micrometer division)	Diameter (μ)	Change in diameter (μ)
		capillary	
normal	2·6	10·4	
acetyl-choline injection	2·7	10·8	+ ·4
post. 8th and 9th stim.	3·1	12·4	+1·6
		arteriole	
normal	6·5	26·0	
acetyl-choline injection	7·1	28·4	+2·4
posterior 8th and 9th stim.	7·1	28·4	0
		venule	
normal	6·7	26·8	
acetyl-choline injection	6·7	26·8	0
posterior 8th and 9th stim.	6·8	27·2	+ ·4

Four similar experiments were made using the plethysmographic method. An example of the result is given in Table IX and Fig. 7. In this experiment the increase in volume caused by the injection of acetyl-choline was 3·5 c.mm. (Fig. 7) and the further increase caused by stimulation of the posterior roots was 4·4 c.mm. The former increase represents that caused by the dilatation of arterioles, accompanied by a slight dilatation of the capillaries. Hence the second increase is less than that caused by the dilatation of capillaries evoked by mere stimulation of the posterior roots, when the capillaries are quite unaffected by acetyl-choline. As concerns the venules, it cannot be decided whether their dilatation is a direct effect of the stimulation of the posterior roots, or only a secondary phenomenon resulting from the dilatation of the arterioles and capillaries.

TABLE IX. Change in volume of a leg caused by acetyl-choline injection and stimulation of the posterior roots (Fig. 7) Frog 37 grams. Room temperature 14° C.

	Time (minutes)	Increase in vol. (c.mm.)		Time (minutes)	Increase in vol. (c.mm.)
	0	0	post. 8th and 9th stim.	40	0
acetyl-choline injection	3	0		42	7·0
	4	1·8		45	7·0
	5	2·6		46	6·2
	6	3·5		52	4·4
post. 8th and 9th stim.	8	3·5		58	2·6
	10	7·9		65	1·8
	12	7·9		71	·9
	13	6·2		78	0
	14	5·3		85	0
	18	3·5			
	24	1·8			
	29	·9			
	35	0			

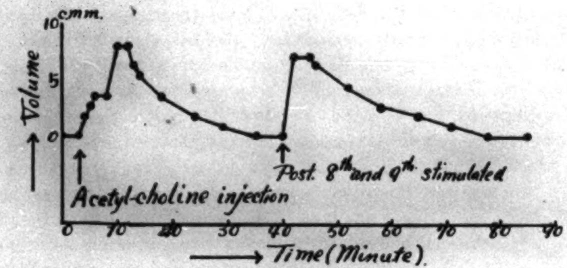

Fig. 7.

CONCLUSIONS.

1. Antidromic fibres are shown to exist in the posterior roots of spinal nerves of the frog.

2. The increase in volume of the hind limb of a frog, caused by stimulating antidromic fibres, is mainly due to dilatation of skin vessels. The same conditions were found by Bayliss to exist in mammals.

3. These antidromic fibres act directly upon both arterioles and capillaries, and cause dilatation of these vessels.

4. In the frog, as in the cat and dog (as proved by Dale and Richards), histamine dilates the capillaries, and acetyl-choline dilates the arterioles both independently of nervous control.

In conclusion I wish to express my thanks to Prof. Starling for his suggestion of this problem and his valuable advice during the course of the work. I also take this opportunity of expressing my thanks to Prof. **Langley** for his valuable advice and kindness in permitting me to repeat some of these experiments in his laboratory.

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ON THE PERMEABILITY OF EPITHELIAL LAYER OF THE BLADDER TO WATER AND SALT. By RINNOSUKE SHŌJI (Japan).

(*From the Institute of Physiology, University College, London.*)

About the end of last century the absorption of various substances from the epithelium of the bladder was studied and discussed. Among many authors **Morro** and **Gaebelein**(1), and **Gerota**(2) may be mentioned. The bibliography before their dates is given in their papers. See also **Hamburger's** book(3). Substances, which were found to pass through the bladder epithelium, were all non-physiological or toxic for the living tissue, for examples, ferrocyanide, alcohol and alkaloids. And, as was pointed out by **Cohnheim**(4) and by **Hamburger**, physiological substances, such as sodium chloride and urea, were applied in an extraordinarily strong concentration. Thus the behaviour of the bladder epithelium under physiological conditions was still obscure. Since **Cohnheim**(4) stated in 1901 that the bladder epithelium was not a diffusion membrane and was impermeable even to water, this problem has been left quite untouched.

The experiments made by **Cohnheim** seemed to be insufficient to support his conclusion, and therefore the present experiments were undertaken at the suggestion of Professor **Bayliss**. The general principle of the experiments is that a saline solution of a certain concentration, which is not far from physiological, is kept in the bladder of a living animal, and the change in concentration of its watery and saline components during various periods of time is determined.

Methods. A rabbit or cat is anæsthetized with ether or ether and chloralhydrate, and laparotonized. Both ureters are tied with double ligatures and cut. A T-cannula is inserted in the neck of the bladder and is tied avoiding blood vessels which run along the neck, in order to keep the circulation in the bladder undisturbed. One branch of the cannula is connected with a burette containing a solution of sodium chloride by rubber tubing. The other branch is also provided with rubber tubing, and serves to drain out the bladder content. The bladder is washed many times with the solution to be tested, which is warmed to body temperature, and then a certain amount of the solution is left in the bladder, both tubes being clamped. At various periods of the experiment, a sample for analysis is taken out by means of an injection syringe, the needle of which is passed through the wall of the rubber tubing into the bladder. The point of the needle is made blunt to avoid any harm if it happens to touch the wall of the bladder, though every possible care is taken not to touch this. The syringe is long (6·3 cm. corresponds to 1 c.c.) and the end of its glass plunger is cut sharply, so that it measures 1 c.c. with an error of at most 2 per mille.

The amount of water absorbed from, or given to, the solution is estimated by measuring the concentration of a colloid contained in the solution. It is clearly necessary that this colloid should not pass through the bladder epithelium, nor undergo any change while it remains in the bladder. It must be measurable quantitatively, and also quite indifferent for the living tissue. It was found that hæmoglobin satisfies these conditions.

The saline solution containing hæmoglobin is prepared as follows: red blood corpuscles of an animal of the same species as that used for experiment are laked with water, and the solution is freed from insoluble stroma and unlaked corpuscles by means of the centrifuge. This solution is added to a NaCl solution, in such proportion that the final solution has the desired concentration of NaCl, and contains, too, about 5 volume p.c. of the water soluble constituents of the corpuscles. To measure the concentration of hæmoglobin, 0·5 or 1 c.c. solution is made up to 25 c.c. with $N/10$ HCl solution, and its colour is compared with a standard solution by means of a Dubosq's colorimeter. The mean of ten readings is taken for each estimation.

That hæmoglobin undergoes no change while it remains in the bladder of a cat or a rabbit is proved by the following experiment. The bladder of a rabbit was filled with 9 c.c. physiological saline solution, and then 1 c.c. solution of 20 volume p.c. of laked corpuscles added to it. After 6 hours the bladder content was washed out thoroughly, and the fluid made up to 200 c.c. containing $N/10$ HCl. Mean reading of colorimeter is 36·1 mm. Next, 1 c.c. of the same hæmoglobin solution was made up to 200 c.c. containing the same amount of salt and $N/10$ HCl. Mean colorimeter reading was 36·2 mm. A similar experiment was made on the cat.

The saline concentration of the solution is determined by chlorine-ion estimation by Volhard's method, using a rather dilute silver solution, 1 c.c. of which corresponds to 2 mg. NaCl, and correspondingly dilute thiocyanate solution. The estimation is made with 0·5 or 1 c.c. solution, and the figure is given in mg. NaCl. The presence of hæmoglobin and other protein from corpuscles in the solution, prepared as described above, does not disturb the estimation of Cl-ion. This is proved by the following experiment.

1 p.c. NaCl solution containing 5 volume p.c. of corpuscles was analysed: (*a*) 1 c.c. solution was diluted to 50 c.c., and its Cl-ion was estimated directly. 10·14 mg. NaCl was found. (*b*) 1 c.c. solution was diluted and its protein was precipitated by heating. Filtrate was made up to 50 c.c., and its Cl-ion was estimated. 10·23 mg. NaCl was found.

Results. Experiments were made with saline solutions of various concentrations between 0 and 4 p.c., and the following results were obtained: when 1 p.c. solution was used, no movement of water and salt was observed; with 0·5 p.c. solution, water was absorbed from the solution, but no movement of NaCl was seen during 6 hours; with 0·25 p.c. and less concentrated solution, both decrease of water and increase of NaCl were found; with 2 p.c. and more concentrated solution, it was always seen that water was given to, and NaCl absorbed, from the solution.

In some of the experiments, the freezing-point depression of the solution was determined at the beginning and end of the experiment, and it was found that the percentage increase or decrease of the freezing-point depression agreed with that of Cl-ion concentration. This means that, not only Cl-ion, but also Na-ion moves with the former. For, if Cl-ion alone move, some other anion ought to come to compensate it, as cations and anions must be always balanced in a solution, and consequently its freezing-point depression ought to be inversely proportional to the dilution of the solution determined by hæmoglobin estimation, and ought not to be proportional to its Cl-ion concentration.

The fact that the permeability of the bladder epithelium thus observed was not due to the action of anæsthetics was proved as follows. A cat was anæsthetized with the least amount of ether and then decerebrated. The animal was kept under vigorous artificial respiration for half an hour in order to drive away any trace of ether from circulating blood, and then similar experiments to those mentioned were made and the same results were obtained.

Twelve experiments were made, of which two are given in the following tables, and will be sufficient to represent the whole. The figures of Table II *a* are calculated from the data of Table II, and show the magnitude of amount of water and NaCl, which is added to or absorbed from the bladder content. The notation used in the tables has the following meaning: the relative dilution, under the column of water, is the ratio of colorimeter reading of the solution at time *t* to that of the original solution, thus it means dilution of the solution at time *t*, that of the original solution being taken as unity. The relative concentration of NaCl is the concentration of the solution at time *t*, that of the original solution being taken as unity. The relative amount of NaCl is the product of the figures of relative concentration of NaCl and of relative dilution of the solution, thus it means a calculated relative concentration of NaCl, the volume of the solution being reduced to its original value.

TABLE I. Experiment with 0·25 p.c. NaCl solution on a rabbit.

	Water		NaCl		
Time (hours)	Colorimeter reading (mm.)	Relative dilution	Amount in 1 c.c. (mg.)	Relative concentration	Relative amount
0	38·2	1·000	2·87	1·000	1·000
1	38·0	·995	2·91	1·014	1·009
2	37·8	·990	2·99	1·041	1·035
3	37·3	·976	3·31	1·153	1·124
4	37·0	·969	3·48	1·212	1·173

TABLE II. Experiment with 2 p.c. NaCl solution on a rabbit.

	Water		NaCl			Freezing-point depression	
Time (hours)	Colorimeter reading (mm.)	Relative dilution	Amount in 1 c.c. (mg.)	Relative concentration	Relative amount	Δ (°C.)	Δ_t/Δ_0
0	28·5	1·000	19·74	1·000	1·000	−1·208	1·000
1	29·3	1·028	18·10	·917	·943		
2	29·7	1·042	16·82	·852	·888		
3	29·8	1·046	15·88	·804	·840		
4	30·0	1·053	15·14	·767	·807		
5	30·2	1·060	14·52	·736	·780		
6	30·3	1·063	14·18	·719	·765	−·880	·728

TABLE II *a*.

	Volume of the bladder content			Total NaCl in the bladder content		
Time interval (hour)	At the beginning of interval (c.c.)	At the end of interval (c.c.)	Gain of water (c.c.)	At the beginning of interval (mg.)	At the end of interval (mg.)	Loss of NaCl (mg.)
0—1	22·00	22·62	·62	434·5	409·5	25·0
1—2	20·62	20·91	·29	373·5	352·0	21·5
2—3	18·91	18·99	·08	318·1	301·5	16·6
3—4	16·99	17·10	·11	269·8	259·0	10·8
4—5	15·10	15·20	·10	228·8	220·8	8·0
5—6	13·20	13·24	·04	191·8	187·8	4·0

The form of the curves of these three figures (relative dilution of the solution, relative concentration and relative amount of NaCl) with respect to time is rather different for each experiment, so that no rule is found, which will express the diffusion velocity of water and NaCl through the bladder epithelium.

Some experiments were made on excised bladders which were immersed in oxygenated Ringer's solution at body temperature. Their permeability was found to be much larger than that of the bladder in a living animal, and moreover it seemed to increase rapidly in a short time after excision, so that the results obtained with an excised bladder cannot be attributed to the physiological property of the bladder.

CONCLUSION.

The epithelial layer of the bladder in a living animal is permeable to water and to NaCl under physiological conditions.

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THE EAR AS MORPHOLOGICALLY AN APPARATUS FOR PERCEIVING DEPTH BELOW SEA-LEVEL.

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THE reason for the close anatomical association of structures so different in their physiological purposes as on the one hand the semicircular canals and otolith-organs, and on the other, the cochlea, has long been a matter for conjecture. The explanation here advanced is that the mammalian organ of hearing subserved in the ancestors of mammals the function of perceiving depth below water surface, and was therefore associated with the semicircular canals and otolith organs in the identification of position and direction of travel.

This hypothesis is supported:—

(*a*) By the close mechanical analogy between the auditory apparatus of mammalia and the depth controlling gear in the naval torpedo, and

(*b*) By the fact that in certain fish (Siluridae) a mechanism is found consisting of ossicles which connects the internal ear and the swim bladder. There is strong evidence that this is used for the perception of depth and not for audition.

Analogy with naval torpedo. In the naval torpedo there are two mechanisms which operate together to control the depth at which the projectile shall travel beneath the surface of the water, (1) a pendulum, (2) a hydrostatic membrane.

The former mechanism under the influence of gravity records any deflection that the long axis of the torpedo may make with the horizontal, and if such a deflection exists it turns the horizontal rudders in such a direction that the deflection tends to be corrected.

The hydrostatic membrane is a sheet rubber diaphragm situated in the side of the torpedo, being in contact with the water on the outside and on the inside with the air-filled interior. If the torpedo is at a depth below the surface the hydrostatic pressure tends to press the diaphragm inwards, but this motion is resisted by a spring. If the torpedo is at the correct depth at which it is required to travel, the hydrostatic pressure produces a force on the diaphragm that is just balanced by the force exerted in the opposite direction by the spring; but if the torpedo gets too deep or too shallow the forces no longer balance and the diaphragm is pressed inwards or allowed to move outwards respectively, and this causes appropriate deflection of the rudders. Experiment shows that either mechanism by itself causes an alternate deflection of the torpedo from the straight line, because of back-lash and friction. But it is found that if both pendulum and hydrostatic membrane operate together, that the one eliminates the tendency to deflection introduced by the other, and the torpedo is thus caused to travel along a straight path at a given distance below the surface.

Applying these conclusions to the fish, it would seem clear that for the proper direction of path in three dimensions of space, at least two mechanisms would be required, namely, one to give information concerning depth below surface, and one to be influenced by gravity, and therefore to show deflection from the horizontal axis. Now the latter mechanism has long been identified as the otolith organ; the hypothesis advanced is that the tympanum, middle ear, and cochlea, are to be identified as the depth recording mechanism.

The tympanum is on this view the counterpart of the rubber hydrostatic membrane of the torpedo. The ossicles are the counterpart of the levers which convey the motion of the diaphragm under varying hydrostatic pressure to the rudder control mechanism, the stapedius muscle acting as the spring which in the torpedo balances the force produced on the diaphragm by the hydrostatic pressure. In fish, the homologue of the cochlea, with its basilar membrane, hair cells, and rods of Corti, is on the above view the mechanism by which the changes in position of the hydrostatic membrane (caused by changes in depth) are perceived by the sending of corresponding stimuli up the cochlea branch of the 8th nerve.

Mechanism of the ear in fish. It is an essential feature of any mechanism for recording changes in hydrostatic pressure, that one side of the diaphragm should be exposed to the fluid pressure (in this case water) while on the other there should be a readily compressible fluid such as a gas or air. And further since this gas or air is liable to be absorbed, there must be means provided of renewing the supply. In the case of shallow water fish and most reptilia, the supply of air in the middle ear may be readily renewed by the fish coming to the surface of the water and causing air to enter *via* the mouth and eustachian tubes. (In some fish the air in the swim bladder is renewed in a similar way(1).) In the case of fish living below the surface, a different method of renewing the air has to be provided, namely by forming a special connection with the gas secreting gland (the swim bladder). This may be effected by either connecting the swim bladder with the middle ear by a tube or duct (Owen(2) says this is the case in many osseous species of fish) or by making the swim bladder itself form the chamber, the change in volume of which (with change of depth below surface) brings about movements of the mechanism which connects with the internal ear. This is the arrangement found in Carp, Loach, and Sleat-fish. About these fish Weber(3) wrote (quoting the abstract given by Owen): "A canal is sent from the sac of each vestibule to a common sinus impar...which communicates on each side by a small orifice with two sub-spherical atria...which atria are supported externally by the ossicles *l* and *m*, and by means of the large ossicle *o* are brought into communication with the fore part of the air bladder *p*. Both the atria and common sinus are filled with endolymph...." Weber actually named these three ossicles the malleus, incus and stapes, and held that they existed chiefly in subserviency to the organ of hearing.

Muller(4) concluded that the air bladder in fishes in addition to other uses serves the purpose of increasing by resonance the intensity of the sonorous undulations communicated from water to the body of the fish. This conclusion is not accepted by Bridge and Haddon(5), who examined the anatomy of the swim bladder in many groups of fish. They state that the ossicles connecting the labyrinth with the swim bladder are less adapted to the conduction of fine sound vibrations than they are to the indications of gross changes in the capacity of the air bladder such as would be brought about either rapidly by external changes in the hydrostatic pressure or slowly by alteration in the volume of gas in that organ by secretion or readsorption. They advance the following points against Weber's theory (that the mechanism has an auditory function): (1) Sound vibrations would be passed from water to the air in the swim bladder with great loss of intensity. (2) In many Siluridae, the walls of the air bladder are very thick, and are therefore ill-adapted for conducting sound. (3) The ossicles have considerable inertia, have no useful lever action, and are not firmly connected (as they are in the ears of mammalia) so that they can vibrate rapidly under the action of sound waves as one concrete mechanism. (4) There is no evidence that the Siluridae, which possess this mechanism, have exceptional powers of hearing.

None of the above objections apply to the alternative view that the mechanism is used for measuring air-bladder volume under changes of hydrostatic pressure. It would seem clear, therefore, at all events so far as this type of mechanism is concerned, that the perception of depth and corresponding control of direction is the function performed and not that of audition.

The intermediate type of mechanism described early in this paper, in which the water exerts its pressure directly on an externally placed tympanum (as it does in the naval torpedo) now comes up for examination. It is at once found that so far as the anatomy and mechanical features of the apparatus are concerned, the function performed could be either that of audition or that of depth perception. And it would seem that both functions could be efficiently performed without modification. If any differentiation were possible, one would say that those fish in which the mechanism appears to be well designed (so far as freedom from friction, inertia losses and back-lash are concerned) are more likely to employ the organ for audition than for depth perception, but this is probably a purely artificial criterion. It is probable then that in this type we see the direct connecting link between the depth perceiving mechanism used by fish, and the apparatus for audition found in mammalia.

SUMMARY.

The view is advanced that the auditory apparatus of mammalia which is represented by the cochlea, fenestræ, ossicles and tympanum once formed in their ancestors, an apparatus for perceiving not sound vibrations, but the depth below sea-level. If this view is correct it would explain why the cochlea, the semicircular canals and the otolith organs are associated together anatomically and have a common nerve, since if the cochlea perceives depth below surface, all three would be directly concerned with the perception of position and the control of direction.

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CARDIAC TETANUS. By W. BURRIDGE, M.B.

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WALTHER(13) gives complete references to the experiments on cardiac tetanus and in his discussion concludes that superposition of contractions and a condition approximating to the tetanus of skeletal muscle is possible in the heart. His experiments were performed on hearts poisoned by muscarine and his illustrations show an incomplete fusion of contractions as the condition resembling tetanus. Röhde(10) also obtained some fusion of contractions in hearts poisoned by chloral hydrate and considered the phenomena as consequent on the drug dissociating the cardiac nervous and muscular elements. His views were confirmed by Carlson(6) and contested by Schultz(11).

The experiments below have been performed on hearts subjected only to alterations in the proportions of their normal environment. They indicate that the greater richness of cardiac muscle in phosphates as compared with skeletal muscle, is an important factor in determining its mode of behaviour.

Method. The hearts were excited through platinum electrodes applied to the base of the ventricles as described in a former paper. The Bayliss frictionless writing point(1) was employed. The hearts were first treated with 5 p.c. potassium chloride, this solution washed out with Ringer's fluid, and then when activity was re-established the fluid replaced by a solution of the composition 0·6 p.c. NaCl, 0·03 KCl, 0·01 p.c. $NaHCO_3$ and *0·15* p.c. $CaCl_2$. This usually caused a temporary increase of tonus and a slight decrease in the height of the spontaneous contractions. See Fig. 1. The reverse change is shown in Fig. 3.

If the heights of the spontaneous contractions were not decreased the treatment with the potassium chloride was repeated. On the other hand in stale hearts the treatment with potassium chloride might not be necessary. Special attention is drawn to the mode of beat of the heart perfused with the solution given, inasmuch as it is similar to the mode of beat described by Ringer(9) as associated with potassium, whereas these hearts were actually perfused with a solution containing large amounts of calcium. This solution of high calcium content perfused through the fresh heart produced the effects described by Ringer as produced by calcium (prolongation of systole, slowing of diastole, etc.). Under the conditions given here, as the illustrations show, it does not. The effects are not consequent on any persisting influence of potassium because a further treatment with potassium, but using the dibasic phosphate instead of the chloride, restored and reinforced the capacity of this calcium solution to produce the effects described by Ringer as produced by calcium. Hence the actions of calcium as described by him are not pure, but the result of calcium interacting with the phosphates abundantly present in the fresh heart. Hence also the effects described in this paper are to be ascribed to chlorides being in excess in the cardiac tissues as well as to calcium being in excess in the perfusing solution.

Fig. 1. 0·15 $CaCl_2$ = perfusion of solution containing 0·15 % $CaCl_2$, 0·6 % NaCl, 0·03 % KCl, 0·01 % $NaHCO_3$. Time every 10″

Von Kries' Experiment (12) was modified to suit a rhythmical preparation. Of the pairs of stimuli used by him, the natural stimulus evoking contraction was taken as the first member and a single induction shock used as the second. By careful watching and practice, the artificial stimulus was applied at gradually decreasing intervals measured from the commencement of the natural contraction. The artificially excited contraction was greater in height according as the interval between it and the natural contraction was less until finally the spontaneous and artificially excited contractions fused into one large contraction. Examples are shown in Fig. 2.

Faradisation produced a summation of contractions in this preparation as regularly as it does in skeletal muscle. Summation of contractions taking place under the influence of calcium has also been recorded by Ringer, Walther, Schultz and others. Examination of the records given by Ringer and Walther shows however that the summation obtained by them was primarily consequent on slowing of the relaxation process. Before summation of contraction in the heart can be directly comparable with the phenomenon given the same name in skeletal muscle, the relaxation process should be of normal duration. Schultz's statement that the compound contractions obtained under the influence of calcium are smaller in amplitude than the single contractions of the unpoisoned heart is incorrect. His error probably arose from the use of damaged tissues (strips of muscle) imperfectly treated by baths. My own experiments performed on hearts of known contractile capacity show that the summated contractions can utilise the whole of the contractile material. The phenomena described by Mays(7) and Mines(8) are probably consequent on loss of phosphates brought about by perfusion(4) or by the acid(5).

Fig. 2. Examples of Von Kries' effect.
Sp. = spontaneous contraction. *S* = contraction excited by a single stimulus.

The preparation described also admits of a complete unwavering tetanus (cf. Fig. 3).

The preparation also showed fatigue. An example is shown in Fig. 4. The fatigue was shown by several phenomena: (1) the refractory period was lengthened as shown by (*a*) replacement of unwavering by wavering tetanus, (*b*) diminution in the height of the tetanic contraction; (2) slowed rate of beat on cessation of stimulus; (3) diminished height of beat. Recovery slowly took place on continued perfusion.

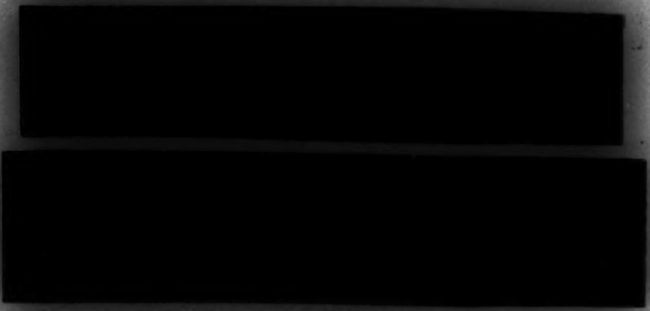

Fig. 3. In the lower tracing note the after shortening and slowing of relaxation after faradisation.

Time every 10 seconds. + =faradisation begun. ↓ faradisation ceased. *S*=contractions evoked by single induced shocks. *N.S.*=Ringer's fluid of normal calcium content ·02 p.c. $CaCl_2$.

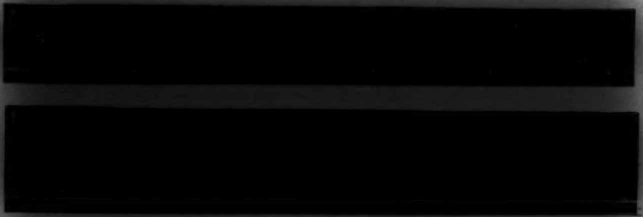

Fig. 4. Previous to *A* the heart had received four periods of faradisation lasting 2½ minutes, as a result of which the height of the faradic contraction had decreased from 25 mm. to that shown here (×½). A complete unwavering tetanus was thereby also changed to the wavering shown in the tracing.

At *NS* the calcium content was decreased to 0·02 % $CaCl_2$. This accelerates the rate of recovery (cf. Fig. 3).

B shows recovery. The rate of beat was subsequently increased by treatment with the dibasic phosphate of potassium.

Time in minutes. *N.S.*=normal Ringer's fluid.

The phenomena in these cardiac cases confirmed my previous results in skeletal muscle that fatigue is primarily consequent on loss of ability to be excited and not on loss of capacity to contract. The potassium chloride method (3) showed that the fatigued hearts maintained an intact stock of contractile material capable of contracting, but of which only a portion could be thrown into action by an induced shock or the normal propagated disturbance. Adrenin had a very marked action in facilitating recovery so that it would appear from this that an alteration in aggregation state (14) may be an important factor in fatigue.

SUMMARY.

1. A cardiac preparation is described which can be (*a*) fatigued, (*b*) thrown into tetanus, and (*c*) show Von Kries' phenomenon.

2. The mode of obtaining the preparation indicates that differing capacities to adsorb different elements of a common environment may be an important factor in determining differences of muscular behaviour.

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NITRITE METHÆMOGLOBIN AND RELATED PIGMENTS.

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IN describing the product formed when sodium nitrite acts on oxyhæmoglobin Haldane points out correctly that not only is its reddish brown colour quite unlike that of ordinary neutral methæmoglobin (*e.g.* prepared by the action of ferricyanide) but also its absorption bands are different in that the α band in the red is less strong in comparison with the β and γ bands than it is in the case of neutral methæmoglobin. Haldane suggested that the increased density of the β and γ bands might be due to the nitrite compound being a mixture of neutral methæmoglobin and an additional pigment. He ruled out the possibility of the additional pigment being oxyhæmoglobin owing to the β and γ bands of the nitrite compound being much more diffuse than those of oxyhæmoglobin, and because saturating the fluid with carbon monoxide changes neither the colour nor the bands. Haldane suggested that the additional pigment might be nitric oxide hæmoglobin because firstly its colour is bright red and therefore any pigment with which it is mixed would be redder than the pigment alone, and secondly its absorption spectra correspond closely in position to those of the β and γ bands of methæmoglobin, and therefore the addition of nitric oxide hæmoglobin would increase the density of these bands relative to the α band and would therefore make neutral methæmoglobin appear spectroscopically like nitrite methæmoglobin. Now if Haldane's suggestion is true, it should clearly be possible to imitate the spectrum of nitrite methæmoglobin by suitable thicknesses and concentrations of ordinary methæmoglobin and nitric oxide hæmoglobin in two separate vessels. This was tested in the following manner. A strong solution of neutral methæmoglobin prepared by the action of potassium ferricyanide on laked blood was dialysed for twelve hours in a collodion bougie against water, so as to remove excess of the unused ferricyanide. This solution was now suitably diluted and divided into three parts.

To part 1, sodium nitrite solution and water were added so as to form nitrite methæmoglobin.

To part 2, sodium nitrite, a trace of ammonium sulphide solution and water was added so as to form nitric oxide hæmoglobin.

To part 3 was added water only so that this was still ordinary methæmoglobin. In each case the amount of water was such that the final volumes of the three parts were the same and therefore each contained finally the same number of hæmoglobin (or derivative) molecules per c.c.

Part 1 was poured into a square trough of 2 cm. thickness. Parts 2 and 3 were placed one in each side of a double wedge trough, the total thickness of which at any point was 2 cm. The square trough and double wedge trough were placed in front of a comparison spectroscope, and the double wedge trough shifted until the bands of the nitrite methæmoglobin and the superposed nitric oxide hæmoglobin and neutral methæmoglobin appeared to be alike. The spectroscope was now removed and the colour examined and it was found that the colours did not match at the point where identical spectra had been obtained, for the nitrite methæmoglobin was too red (or the superposed pigments too brownish yellow) for the colours to match. If the wedge trough were shifted until the colours matched, then the spectroscope showed that the bands no longer appeared alike since the β and γ bands of the superposed pigments were too dense to match with those of the nitrite methæmoglobin. Now since the light encounters similar thicknesses and concentrations of hæmoglobin derivatives and ferricyanide when passing through the square and the double wedge troughs, but dissimilar amounts of sodium nitrite (since it was not added to the ordinary methæmoglobin) and ammonium sulphide (since it was added only to the nitric oxide hæmoglobin), it would seem possible that one of these two photometrically unbalanced reagents is responsible for the want of agreement between the nitrite methæmoglobin and the mixture of ordinary methæmoglobin and nitric oxide hæmoglobin. But of these two reagents one, namely sodium nitrite, is colourless and is therefore not likely to be the cause of the want of agreement. So that the possible disturbance produced by the ammonium sulphide remained to be eliminated. This was done by treating a small quantity of a strong solution of nitrite methæmoglobin with sufficient dilute ammonium sulphide in the warm to completely form nitric oxide hæmoglobin. This strong solution was now dialysed against water in a collodion bougie and was then diluted till colorimetrically it had the same strength as the nitric oxide hæmoglobin previously used. The two solutions hardly differed in colour, but the new solution was slightly pinker than the other owing to the reduction in the amount of free ammonium sulphide. Some of this new solution was placed in one side of the double wedge trough, the other being filled as before with ordinary methæmoglobin and the square trough being filled as before with the nitrite methæmoglobin. Comparison of the spectra and the colours showed that there was still the same want of agreement, *i.e.* when the spectra were similar, the mixture in the double wedge trough was found to be too brownish yellow. This want of agreement might be explained in two ways, either by the presence in nitrite methæmoglobin of some additional pigment (or pigments) beside nitric oxide hæmoglobin, or by nitrite methæmoglobin not being in point of fact a mixture at all. With regard to the first possibility, it is clear that the additional pigments must have two properties in order that it should cause suitable modification when added to ordinary methæmoglobin, firstly its absorption spectra must closely resemble the β and γ bands of nitrite methæmoglobin, and secondly its colour must be pinker than that pigment. The only other derivative of hæmoglobin having the required properties (beside nitric oxide hæmoglobin that had already been tried above) was found to be alkaline methæmoglobin, for colorimetrically this pigment is pinker than nitrite methæmoglobin and spectroscopically its β and γ bands are very similarly situated as the following table shows :

α	β	γ	Pigment
(6040)	5779	5406	Alkaline methæmoglobin
(6311)	5770	5390	Nitrite methæmoglobin
	α	β	
(——)	5786	5412	Nitric oxide hæmoglobin

The above values are in Ångstrom units ($=10^{-8}$ cm.).

Now in order that alkaline methæmoglobin should be a constituent of nitrite methæmoglobin, it must be able to exist side by side with ordinary neutral methæmoglobin without chemical change occurring. To ascertain if this is possible a solution of ordinary methæmoglobin was gradually titrated with N/100 soda, the spectroscope being used to watch the changes that took place. It was found as the neutral methæmoglobin changed to alkaline methæmoglobin that the α band of the former gradually faded to be replaced by the α band of the latter, the β and γ bands suffering but slight change. There is therefore a gradual transition of one pigment into the other as the alkali is added, and therefore any mixture of the two pigments could exist in a stable state. The hydrogen ion concentrations of some of the mixtures was determined approximately by mixing small quantities of neutral methæmoglobin with excess of different buffered solutions which formed a series from 6 to 10 P_H. The mixtures were then compared spectroscopically with solutions of neutral and alkaline methæmoglobin placed one on either side of a double wedge trough. The results are shown in the table below:

P_H	Percentage of alkaline methæmoglobin
7	0
8	0–5
8·2	5–10
8·4	about 25
8·6	55–60
8·8	about 75
9	95–100
10	100

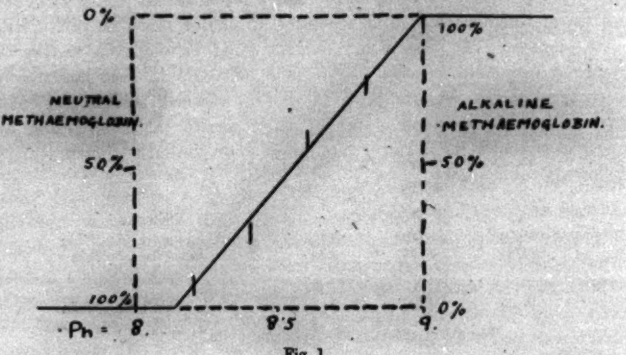

Fig. 1.

These values are shown plotted in Fig. 1. The graph shows that neutral methæmoglobin exists in solutions on the acid side of P_H 8, alkaline methæmoglobin on the other hand exists in solutions on the alkaline side of P_H 9. Between these two values the various mixtures of alkaline and neutral methæmoglobin exist together in the various proportions shown in the table. That is methæmoglobin behaves like an indicator for the range P_H 8 to P_H 9.

Comparison was made between the bands of nitrite methæmoglobin and different mixtures of neutral and alkaline methæmoglobin in the double wedge trough and it was found when the spectra and colours of the solutions otherwise match, that the α band of alkaline methæmoglobin (at 6040 A.U.) acts as a disturbing factor. Nitric oxide hæmoglobin was now placed in a separate trough, and the beam of light that passed through the double wedge trough was caused to pass first through this trough as well, so that different thicknesses of neutral methæmoglobin, alkaline methæmoglobin and nitric oxide hæmoglobin could be matched with the nitrite methæmoglobin. It was found that the colour and spectra of the latter could be matched if a sufficient thickness of the alkaline methæmoglobin was used for its α bands (at 6040 A.U.) to be just visible. Since, however, nitrite methæmoglobin shows no band in this position, it would appear impossible to imitate the colour and spectra of nitrite methæmoglobin by means of mixtures of the three pigments. If, therefore, nitrite methæmoglobin is in fact a mixture of pigments, it is difficult to ascertain what the pigments present can be. The attempt was now made to vary the strengths of different pigments by the action of dilute alkali, dilute acid, heat coagulation, and dialysis against water in a collodion bougie. The spectra of nitrite methæmoglobin did not, however, undergo any change that would indicate any variation in the composition of the solution, such as would be expected if a mixture of pigments of different chemical composition was present. Thus alkali would leave nitric oxide hæmoglobin unchanged, but would cause all the methæmoglobin present to change to the alkaline phase, the end product would therefore be a mixture of this pigment and nitric oxide hæmoglobin. When, however, comparison was made between ordinary alkaline methæmoglobin and nitrite methæmoglobin treated with alkali, the colour and spectra appeared to be identical, showing no nitric oxide component to be present.

As experiment had given entirely negative evidence for the composite nature of nitrite methæmoglobin tests were commenced to find proof for its existence as a definite chemical compound. For this purpose the well-known effect of ammonium sulphide in reforming oxyhæmoglobin from methæmoglobin (both neutral and alkaline) was used. A solution of laked blood was divided into two equal parts, one part was fully saturated with CO gas, the other part was treated with sodium nitrite to form nitrite methæmoglobin. The latter was also fully saturated with CO gas and of course underwent no change either colorimetric or spectroscopic. To this a solution of ammonium sulphide was added thus reconverting all methæmoglobin present into oxyhæmoglobin, which in the presence of CO gas would be converted into carbon monoxide hæmoglobin and would stop at that point, since the ammonium sulphide is not able to bring about the reduction that it effects when carbon monoxide gas is not present in the solution. Now Gamgee(2) found with the ordinary spectroscope that the bands of nitric oxide hæmoglobin are identical in their positions with those of oxyhæmoglobin; measurements with the reversion spectroscope(3) show that there is a considerable difference between the positions of the bands of the three compounds of hæmoglobin. The values obtained are given in the following table:

	α band A.U.	β band A.U.
O2Hb	5768	5398
COHb	5714	5360
NOHb	5785	5418

Experiments with the double wedge trough showed that a gradual change in the position of the α band in the spectrum occurs from 5714 A.U. to 5785 A.U. as carbon monoxide hæmoglobin is displaced by nitric oxide hæmoglobin. By ascertaining the wave length of the α band by means of the reversion spectroscope the relative amounts of the CO and NO compounds can be accurately ascertained. It was found further that as little as 6–8 p.c. NOHb could be recognized in a solution of the two pigments. By changing the methæmoglobin compounds in nitrite methæmoglobin into carbon monoxide hæmoglobin by the reaction above described any nitric oxide present in greater quantity than 6–8 p.c. would at once disclose itself by shifting the α band. Now measurements had shown that if nitrite methæmoglobin were a mixture of methæmoglobin and nitric oxide hæmoglobin as Haldane supposed at least 36–40 p.c. of the latter would have to be present in order to give the colour and absorption spectra that nitrite methæmoglobin is found to possess. Having treated some nitrite methæmoglobin with CO gas and ammonium sulphide, the α bands were measured and found to be in their normal position, and therefore less than 6–8 p.c. nitric oxide hæmoglobin must have been present, that is roughly 30 p.c. less than Haldane's mixture would require. This is therefore definite evidence for nitrite methæmoglobin being a definite chemical compound.

SUMMARY.

The following evidence is in favour of nitrite methæmoglobin being a definite chemical compound:

(1) Mixtures of certain pigments in different proportions did not match the colour and spectra of nitrite methæmoglobin.

(2) Reagents which would be expected to act differently on various constituents if these were present, and therefore to modify the appearance of the absorption bands, were not found to bring about such a change.

(3) Nitric oxide hæmoglobin is not a constituent, for if nitrite methæmoglobin be converted into carbon monoxide hæmoglobin by the action of CO gas and ammonium sulphide, the a bands are found to occupy their normal positions.

(4) Methæmoglobin has been found to behave like an indicator over the range P_H 8–9. Below 8 methæmoglobin shows the spectra and colour of "neutral" methæmoglobin, above 9 on the other hand those of "alkaline" methæmoglobin are given. Between 8 and 9 both pigments exist side by side in the solution, their relative amounts varying with the reaction.

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VARIATIONS IN THE RESISTANCE OF PROTOZOON ORGANISMS TO TOXIC AGENTS. By R. A. PETERS, M.D., *Fellow and Lecturer of Gonville and Caius College.*

(From the Biochemical Laboratory, Cambridge.)

In a research upon the course of the disinfection of cultures of bacteria by various disinfectants, Chick (1) came to the conclusion that there was in most cases a close analogy between the disinfection considered as a reaction, and the course of a "unimolecular" chemical reaction. If the disinfection process was plotted as a graph in which the abscissæ represent time, and the ordinates the logarithm of the percentage of survivors, the resulting points lay upon a straight line. The experiments have received some criticism upon the lines that the course of the reaction really represents differences of resistance to the toxic agent (2). Others (3) are of opinion that the experiments mean that bacteria show no variations in resistance. It seemed to be of value to test the point upon some cultures of the ciliate protozoon colpidium. These organisms can be obtained in sufficient numbers to rule out statistical differences.

The method of experiment used was to take 1 c.c. of a culture of colpidia (concentrated if necessary by slow centrifuging) and to add to it carefully drops of a M/1000 $HgCl_2$ solution from a capillary pipette, noting the time of addition. At given intervals after the addition, one drop was withdrawn from the mixture with another capillary pipette and added respectively to a small drop of potassium ferrocyanide, M/100, previously placed upon a microscope slide. Potassium ferrocyanide is comparatively non-toxic to the colpidium and serves to remove the traces of mercury which would otherwise continue to act during the process of counting. The number of organisms already dead were quite easily counted as they fall to the bottom of the drop. The counts were made as soon as possible after removal from the mixture, using a mechanical stage and 1 inch objective. After the count a trace of solid $HgCl_2$ was added to the drop. This quickly killed and fixed the remaining colpidia so that the total number of organisms in the sample withdrawn could be counted.

Experiments 1 and 2, Table I (Figs. 1, 2), show two such experiments. They are illustrations of a case in which it took (1) about 50 minutes to kill all organisms, and (2) about 12 minutes. Column 1 gives the time of withdrawing the sample, column 2 the number dead, column 3 the total number in the sample. Column 6 is the logarithm of the percentage of survivors. The diagram (Figs. 1, 2) shows the results. The curves *B* show the results plotted as the logarithm of the percentage of survivors. It will be seen that there is first a kink after which the relation between the time and the log. percentage of survivors is a straight line. The results imitate those obtained by Chick closely. In some of her later experiments(4), she obtained an identical kink to that found in the case of colpidium curves. There would seem then little doubt that the same type of process is being observed in both Chick's experiments and my own. As Brooks(2) points out the result can be got by assuming nothing more fundamental than variations in resistance to the toxic agent. Let us consider the question from first principles.

A priori, we should expect to find variations in the resistance of bacteria or protoza in any culture to any toxic agent. For instance in a culture of paramœcia, even if grown from the same original paramœcium, we find marked variations in the length of the organisms. Jennings(5) has worked out frequency curves for such variations. Suppose that it was easier for the toxic agent to penetrate a small colpidium than a larger one, we should at once get a relation for the time of death based upon a frequency curve.

TABLE I.

Exp. 1. Fig. 1 *a*. 1 c.c. colpidium culture + 7 drops $HgCl_2$ M/1000*. Temp. 16·7° C.

Time	Dead	Total removed	p.c. died	p.c. surviving	$\log_{10}$ survivors
5 mins.	7	230	30	97	1·99
10 „	15	277	5	95	1·98
15 „	63	190	33	67	1·83
20 „	118	231	51	49	1·69
25 „	165	233	71	29	1·46
30 „	247	325	76	24	1·38
35 „	192	221	87	13	1·11
48 „	12	298	96	4	·6

Exp. 2. Fig. 1 *b*. 1 c.c. colpidium culture + 5 drops $HgCl_2$ M/1000. Temp. 15° C.

Time	Dead	Total removed	p.c. died	p.c. surviving	$\log_{10}$ survivors
1 min.	3	345	1	99	1·996
3 mins.	71	337	21	79	1·9
5 „	165	307	54	56	1·75
6 „	223	380	59	41	1·61
7 „	277	365	76	24	1·38
8 „	342	411	83	17	1·23
11 „	1 alive	—	100	0	

* The amount of $HgCl_2$ requiring to be added varies according to the condition of the culture. We are not concerned here with the absolute toxicity of $HgCl_2$, but merely with the rate at which the death process proceeds.

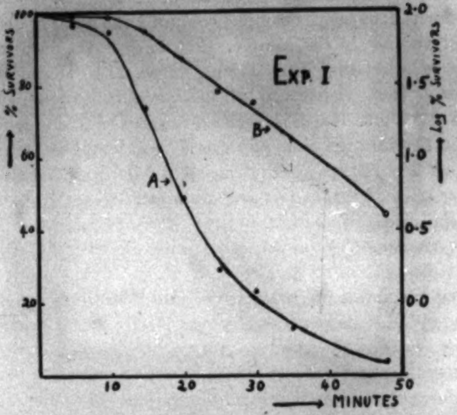

Fig. 1. Death curve for colpidia, Exp. 1. (*a*) Ordinates p.c. survivors. (*b*) Ordinates $\log_{10}$ p.c. survivors.

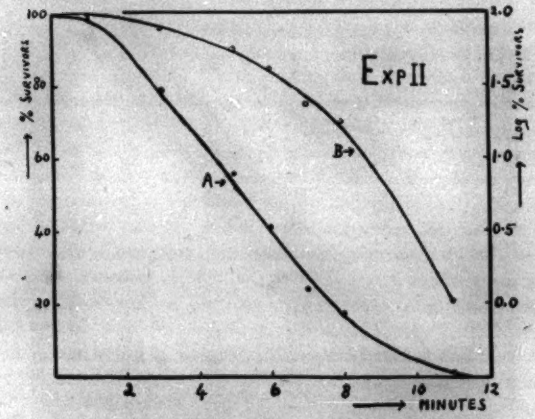

Fig. 2. Death curve for colpidia, Exp. 2. (*a*) Ordinates p.c. survivors. (*b*) Ordinates $\log_{10}$ p.c. survivors.

We may estimate the resistance of a given organism by the time taken to kill that organism with a given toxic agent. The majority of the organisms in the culture will have a certain mean resistance, some will die quicker and some slower than the greatest number. This can be represented by an ideal frequency curve (Fig. 3), Yule(6).

The area included between the curve and the x axis represents the total number of organisms in the culture. The value of x represents the resistance of the organisms (as measured by the time taken to kill). The value of y represents the number of organisms out of the whole culture having a resistance of x.

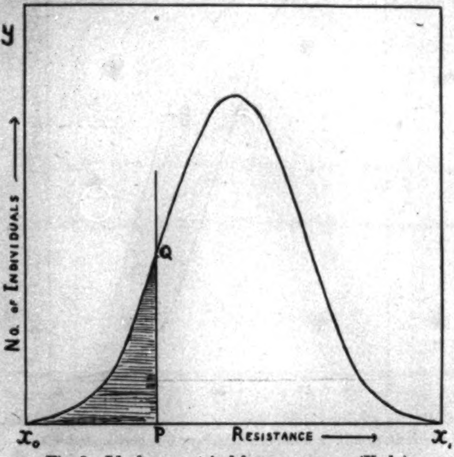

Fig. 3. Ideal symmetrical frequency curve (Yule).

In the case of a frequency curve plotted upon squared paper, the number of small squares included between the curve and the x axis will represent the number of individuals in the culture. Suppose that we submit the culture to the action of a killing agent, the killing agent may be represented by a straight line sweeping from $x_0 \rightarrow x_1$ parallel to the axis of y. It will wipe out successive portions of the culture starting with the least resistant. If we stop the killing process at any point P, the shaded area x_0PQ will represent the number of individuals killed. By counting the number of squares in this shaded area and dividing by the total number of squares representing the whole culture, we can obtain the percentage of individuals which have died up to the point *P* and so the percentage of survivors. Proceeding in this way we obtain a series of figures which are the percentage of survivors at different times. These may now be plotted as the ordinates of another curve, curve A, Fig. 4, which is readily recognized as the S-shaped statistical curve. If we plot the logarithm of the percentage of survivors, we get curve B, Fig. 4. The latter curve it will be seen bends rapidly down and becomes practically a straight line.

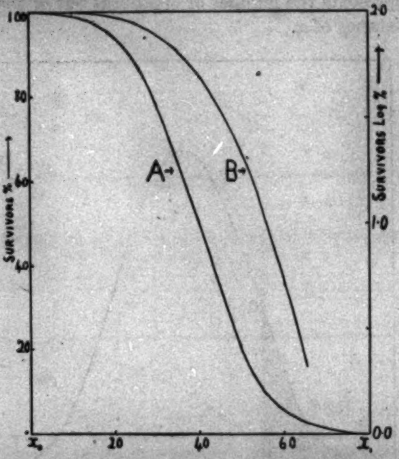

Fig. 4. *A*. Ordinates p.c. survivors. Abscissae. Resistance of individuals, measured in terms of the time to kill. *x* units are arbitrary but correspond with *x* units of Fig. 3. *B*. Ordinates $\log_{10}$ p.c. survivors. Abscissae as for *A*.

If these curves (Fig. 4) are compared with Figs. 1, 2, obtained from killing colpidium cultures, it will be seen that the general resemblance is very striking. The only real difference is that the S-shaped curve for the colpidia is not symmetrical. This is however the rule for biological material, and means that the frequency curve is not an ideal one, but rather of the nature of Fig. 5 (7), a skew-shaped curve. The S curves and the corresponding logarithmic curves have been calculated and are shown (Fig. 6 (1, 2)). Variation will then explain quite well Miss Chick's results for Staphyloccus pyogenes aureus. The curves which are straight lines from the beginning may well be explained as follows. If we take a toxic agent sufficiently strong to kill a number of the colpidia quickly before the first observation we shall not get any observations of the first part of the curve at all. The protozoon curves confirm the suggestions made by **Brooks** upon this point.

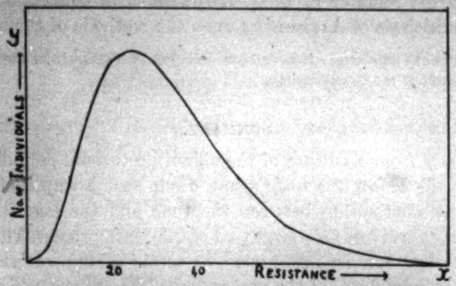

Fig. 5. Asymmetrical frequency curve (skew-shaped) (Yule).

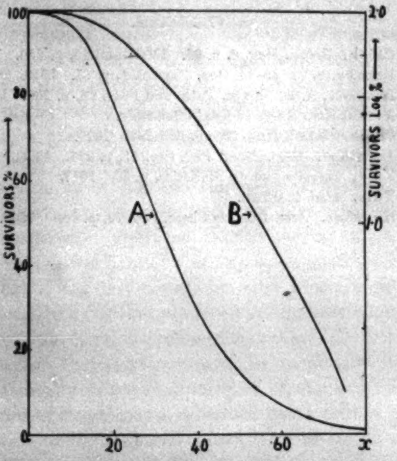

Fig. 6. *A*. Ordinates p.c. survivors. Abscissæ. Resistance of individuals, measured in terms of the time to kill. x units are arbitrary but correspond with x units of Fig. 5. *B*. Ordinates $\log_{10}$ p.c. survivors. Abscissæ as for *A*.

It seems then that the logarithmic death shows that the bacteria and colpidia exhibit variations in resistance to any toxic agent. For purposes of disinfection the monomolecular law is an admirable working rule provided that it remains as such and does not involve the idea that bacteria can be treated as single chemical molecules. The reader is referred to the remarks of F. G. Hopkins(8) in connection with the sweeping criticisms of Arrhenius upon the methods of the biologist.

The expenses of this research have been defrayed by a grant from the Government Grant Committee of the Royal Society.

SUMMARY.

Organisms from a culture of the ciliate protozoon, colpidium, when exposed to the action of a toxic agent, die in such a way that there is a straight line relationship between the time and the logarithm of the percentage of survivors for a large part of the death process. The apparent logarithmic law can be interpreted in terms of the variation in the resistance of individuals of the protozoon culture to the toxic agent.

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THE ACTION OF CERTAIN SERUM CONSTITUENTS UPON THE HEART AND PLAIN MUSCLE. BY A. J. CLARK.

(From the Pharmacological Departments of Guy's Hospital and University College, London.)

IN a previous paper (1) I showed that serum produced a slight augmentor effect upon the freshly isolated heart of the frog, and a very strong augmentor effect upon the frog's heart, after prolonged perfusion with Ringer's fluid (hypodynamic heart). I also showed that this effect was due entirely to the alcohol soluble constituents of the serum, and that lecithin and even soaps of the higher fatty acids produced similar effects.

Numerous workers have studied the action of serum upon the isolated organs of the frog and the mammal, and some of the most important results are as follows. O'Connor (3) found that serum produced vaso-constriction in the perfused frog, and that in isolated mammalian tissues, serum produced increased contractions of the uterus, gut and bladder, and vaso-constriction, he found moreover that hirudin plasma produced none of these effects. Cushny and Gunn (2) found, with the isolated heart of the rabbit, that the addition of serum to the perfusion fluid caused (*a*) a preliminary stage of stimulation in which the contractions were augmented and usually accelerated and (*b*) a subsequent stage of depression in which the contractions became progressively weaker and slower. They showed that this depression was due to the intense vaso-constriction produced by the serum, which stopped the flow of fluid through the heart. Yanagawa (4) confirmed these results and showed that if the serum proteins were precipitated by acetone-ether, then the protein fraction produced vaso-constriction, whereas the acetone-ether soluble fraction produced no effect.

The results show that the reaction of the mammalian heart to serum constituents is totally different from that of the frog's heart. I performed the series of experiments described below with a view to determining the action of the different constituents of serum upon different tissues.

Preparation of material. Sheep's serum (800 c.c.) was rapidly dried on glass plates in a current of air. The dried powder was emulsified with 200 c.c. water and poured into 1000 c.c. of ice cold alcohol. The precipitate was washed with alcohol and with ether and then dried (fraction I containing the serum proteins). The alcohol-ether fractions were mixed, evaporated to 100 c.c., and an equal volume of water was added, and the turbid fluid was shaken with successive fractions of ether, the ether extracts were finally evaporated to dryness (fraction II containing the substances soluble in alcohol and ether). At the same time a sample of moderately pure lecithin was prepared by extracting a sample of lecithin with acetone and water and taking up the residue with absolute alcohol.

Experiments upon frog's tissues. The following four substances were tested: serum, the two serum fractions, and lecithin. These substances were tested upon (i) isolated hearts of R. temp. set up in the method described in a previous paper(1), and (ii) upon the vessels of frogs perfused by the Trendelenburg method. The results obtained are summarised in Table I, all the figures represent the average of several observations.

Table I. The figures denote the percentage alterations observed ten minutes after the introduction of the substance under investigation.

	I (hypodynamic) frog's heart.		II Rate of flow through vessels of perfused frog
	Amplitude of contraction	Frequency	
Serum 0·5	+ 80	+20	−10
2·0	+100	+15	−50
5·0	+100	+50	
Serum proteins 0·2	nil	nil	−40
0·5	nil	nil	−50
1·0	nil	nil	−85
Alcoholic extract of serum 0·001 ...	+ 20	nil	nil
0·005 ...	+400	+50	+30
Lecithin 0·0001	+ 50	+30	nil
0·001	+100	+80	nil
0·005	—	—	+10

Experiments were also made with plasma from defibrinated blood and with dried serum suspended in water, the results obtained were the same as with fresh serum.

These results show that the vaso-constrictor action of serum upon frog's vessels is due to those constituents which are precipitated by alcohol (probably the serum proteins), whereas the augmentor action of serum upon the frog's heart is due to the alcohol soluble substances; these latter substances have a slight vaso-dilatator action (cf. Fig. 1).

Experiments were also performed to determine the action of serum upon the movements of the frog's gut. Frogs were perfused through the left aorta, the viscera were exposed, and records were taken of the movement of the stomach muscle, and of the rate of flow of the perfusion fluid. Serum, alcoholic extract of serum, and lecithin all produced increased tonus of the stomach muscle, this effect is shown in Fig. 1; the serum proteins however produced no such effect.

Fig. 1. Frog perfused through aorta. Movements of stomach (upstroke systole), also rate of flow of fluid (drop recorder). I. Effect of addition of serum 0·5 p.c. to perfusion fluid. Increased tonus of stomach and vaso-constriction. II. Effect of addition of lecithin 0·01 p.c. to perfusion fluid. Increased tonus of stomach and vaso-dilatation.

Experiments upon the rabbit's heart and blood vessels. The action of serum and its derivatives was determined upon (i) isolated rabbit's hearts perfused through the aorta, (ii) isolated rabbit's auricles, which were cut off from the rest of the heart and suspended in Ringer's fluid through which a free stream of oxygen was passed, and (iii) rabbit's ears perfused through the artery.

The effect of serum upon the rabbit's heart is shown in Fig. 2, this shows the result described by Cushny and Gunn, namely an initial increase in the force of the beat and then great vaso-constriction, followed by diminution in the rate and force of the beat; when however the auricles were removed from the same heart and suspended in Ringer's fluid, the addition of serum caused a slight increase in the force of the beat, and no alteration in the rate, and after prolonged isolation of the auricle, the addition of serum caused a well-marked increase in both the frequency and the force of the beat. This shows that the deleterious action of serum upon the perfused heart is due to its action in producing vaso-constriction. The effects of serum, and of serum derivatives, and of lecithin, upon the auricle, the blood vessels, and the perfused heart of the rabbit are summarised in Table II.

Table II shows that serum and serum proteins cause strong vasoconstriction whilst the alcoholic extract of serum causes slight vasodilatation, this result agrees with the results obtained by Yanagawa(4).

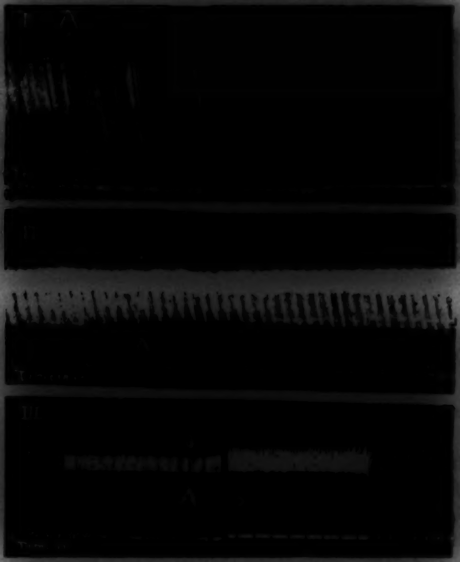

Fig. 2. I. Isolated rabbit's heart perfused with Ringer. Temp. 35° C. Movements of ventricle recorded. At *A* 10 c.c. of Ringer containing 10 p.c. rabbit's serum injected into side tube. Previous to injection. Frequency 100 per minute. Rate of flow of perfusion fluid, 8 c.c. per minute. Two minutes after injection. Frequency, 28 per minute. Flow of fluid arrested.

II. Auricles removed from the heart and suspended in 50 c.c. of Ringer. Temp. 35° C. At *A* 1 c.c of serum added to bath. Frequency before addition of serum, 196 per minute; frequency 5 minutes after addition, 174 per minute.

III. Same preparation as in II, 5 hours later, suspended in Ringer in intervening period. At *A* 1 c.c. serum added to bath. Frequency before addition of serum, 90 per minute. Frequency 5 minutes after addition, 138 per minute.

Serum also causes a slight increase in the force of contraction of the isolated auricle, serum proteins have no action on it and alcoholic extract of serum and lecithin have no augmentor action and if anything depress its activity. The striking augmentor effect produced by serum, alcoholic extract of serum and by lecithin upon the frog's heart is not shown by the mammalian heart.

In a previous paper I advanced the hypothesis that the hypodynamic condition produced in the frog's heart by prolonged perfusion was due to a loss of lipoids, and that this was the reason for the strong action produced by lipoid substances upon the hypodynamic heart. The mammalian heart after prolonged perfusion becomes very enfeebled, but in this case serum has a relatively weak augmentor action and serum lipoids have no action at all. The weakening of the mammalian heart is therefore due to causes other than those producing weakening of the frog's heart. I believe that the chief cause for the failure of the mammalian heart is due to insufficient oxygen supply, and that this masks any other effects.

TABLE II. The figures denote the percentage alterations observed five minutes after the introduction of the substance under investigation.

	Perfused rabbit's heart			Isolated auricle of rabbit		Perfused rabbit's ear
	Frequency	Amplitude of contraction of ventricle	Rate of flow of fluid	Frequency	Amplitude of contraction of auricle	Rate of flow of fluid
Serum 0·2 ...	−10	+ 5	−10	nil	nil	−40
1·0 ...	−20	−20	−60	nil	+10	−45
2·0 ...	—	—	—	nil	+20	—
Serum proteins 0·05	—	—	—	—	—	−10
0·1	− 5	−40	−40	nil	nil	—
0·2	—	—	—	—	—	−40
0·5	—	—	−95	nil	nil	—
Alcoholic extract of serum 0·01	−10	−15	+20	−6	−20	+ 6
Lecithin 0·002 ...	−10	nil	−20	nil	nil	—
0·005 ...	—	Heart arrested	—	nil	nil	−10

There are indications that after prolonged isolation the mammalian auricle suffers changes similar to those observed in the frog's heart. The isolated auricle even after prolonged isolation continues to give up to the surrounding fluid some substance which lowers surface tension, moreover serum produces a distinctly greater effect upon the long perfused than upon the fresh auricle.

I noted also that in the hypodynamic frog's heart changes in the ionic content in the Ringer's fluid produced a much greater effect than did the same changes in the fresh heart, and I attributed this to the loss of lipoid having made the cells more permeable. A similar effect is seen with the isolated mammalian auricle. Fig. 3 shows the effect of increasing the percentage of sodium chloride in the Ringer's fluid upon the fresh and the exhausted mammalian auricle, and it will be seen that the effect is much greater in the latter case.

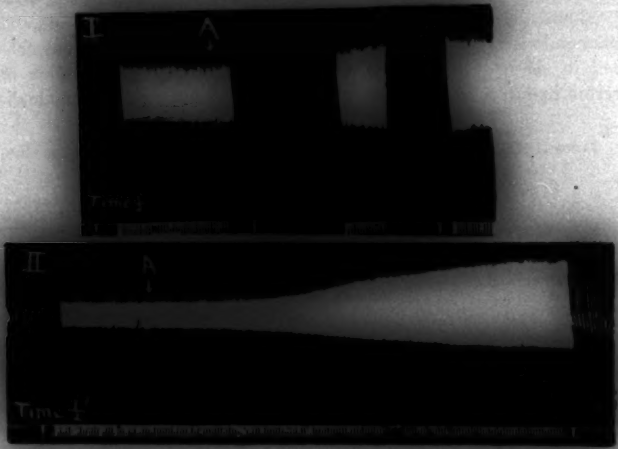

Fig. 3. I. Auricle 30 minutes after isolation in normal Ringer's fluid (NaCl ·9 p.c.; $CaCl_2$ ·012 p.c.; KCl ·04 p.c.; $NaHCO_3$ ·02 p.c.). At *A* a fluid was substituted containing half the normal quantity of sodium chloride with cane sugar added to render it isotonic (NaCl ·45 p.c.; cane sugar 4·8 p.c.; other constituents as before).

II. Same auricle 3½ hours later, suspended in normal Ringer's fluid during intervening period. At *A* fluid containing NaCl ·45 p.c. and cane sugar 4·8 p.c. substituted for the normal fluid.

Experiments upon the isolated gut and uterus of mammals. The actions of serum, serum derivatives and of lecithin upon the isolated gut of rabbits, and the isolated uterus of guinea pigs, are shown in Table III.

Table III shows that serum produces a marked increase in the activity of the isolated gut and uterus, and that the alcoholic extract of serum and lecithin produce similar effects but that serum proteins produce no effect.

TABLE III. The figures denote the percentage alteration in activity after five minutes exposure to the substance under investigation.

	Isolated gut of rabbit		Isolated uterus of guinea pig
	Frequency of contraction	Height of contraction	
Serum 0·1	+10	+20	—
0·5	+10	+25	—
5·0	—	—	Increased systolic tonus
Serum proteins 0·1	nil	nil	—
0·5	nil	nil	nil
Alcoholic extract of serum 0·001	+ 5	+18	—
0·005	+ 5	+24	—
0·01	+ 5	+50	increased systolic tonus
Lecithin 0·01	+ 5	+ 5	Increased systolic tonus

DISCUSSION AND SUMMARY.

The actions of serum and of serum derivatives upon the isolated tissues of the frog and mammal are summarised in Table IV.

TABLE IV.

+ indicates vaso-constriction, increased tonus, or augmentor effect.
– indicates vaso-dilatation.
○ indicates no action.

	Serum	Serum proteins	Alcoholic extract of serum
Frog's tissues			
i. Heart, recently isolated	+	○	+
ii. Heart, hypodynamic	+ + +	○	+ + +
iii. Blood vessels	+ +	+ +	–
iv. Stomach	+	○	+
Mammalian tissues			
i. Isolated auricle	+	○	○
ii. Blood vessels	+ + +	+ + +	–
iii. Gut	+ +	○	+ +
iv. Uterus	+	○	+

The results set out in Table IV show that the action of serum upon isolated tissues is the sum of the action of the serum proteins and of the alcohol soluble constituents and that these two sets of substances have totally distinct actions.

The serum proteins produce strong vaso-constriction and have little action upon other tissues, while the alcohol soluble substances produce slight vaso-dilatation, a great augmentor effect upon the frog's heart, a moderate augmentor effect upon the frog's stomach, and the mammalian gut and uterus, but no effect upon the mammalian auricle.

Probably other substances such as adrenalin and histamine are often concerned in the action of serum or of tissue extracts upon isolated organs, but the mode of preparation of the serum derivatives used in the above experiments must have removed most of these substances.

The action of the alcoholic extract of serum and of lecithin in stimulating plain muscle indicates that an alcoholic extract of any tissue is likely to cause stimulation of plain muscle, and that this general action must be carefully distinguished from the specific action of extracts of special glands such as pituitary extract.

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The expenses of this research were defrayed in part by a grant from the British Medical Association.

THE EFFECT OF ALTERATIONS OF TEMPERATURE UPON THE FUNCTIONS OF THE ISOLATED HEART.

By A. J. CLARK.

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Several investigators have determined the effect of alterations of temperature upon the isolated hearts of frogs and mammals. Much of this work was done to determine whether the frequency of the heart was a linear or logarithmic function of the temperature. Other workers determined the coefficient of the alteration in frequency when the temperature was varied 10° C., and tried to determine whether this coefficient was a constant over the full range of temperature at which the heart would function, and also whether the temperature coefficient corresponded to the coefficient of a chemical or of a physical reaction. Flatow(3) and Snyder(8) investigated the effect upon the isolated frog's heart of alterations of temperature, and both found that the frequency was not a linear function of the temperature. Knowlton and Starling(5) investigated the effect of alterations of temperature upon the isolated mammalian heart and found that the relation between frequency and temperature was a linear one, but they expressly stated that they considered that this relation was probably accidental. Snyder(9) found a non-linear relationship between the frequency and the temperature in isolated hearts of cats and of dogs and Frank(4), who varied the temperature of intact rabbits and measured the frequency at different temperatures, obtained results which also show a non-linear relationship.

The action of temperature upon the frequency of the frog's heart. I measured the effect of variations of the temperature upon the isolated heart of the frog by means of the perfusion apparatus shown in Fig. 1. The apparatus was submerged in a water bath; the temperature of the fluid passing through the heart could be easily fixed at any desired point. The average of six experiments upon the heart of Rana temporaria in winter in England, is shown in Fig. 2, curve I, and a sample protocol of an experiment is given in Table I. The curve agrees with the results obtained by Flatow(3) and Snyder(8) and shows that the frequency is not a linear function of the temperature.

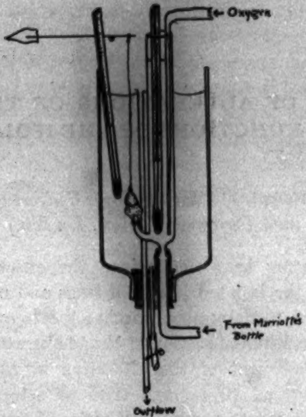

Fig. 1.

Table I. 10.2.20. R. temp. isolated heart perfused with normal Ringer. (NaCl 0·65 %, KCl 0·014 %, $CaCl_2$ 0·012 %, $NaHCO_3$ 0·02 %, P_H – 8·0.)

Time	Temp.	Rate	Time	Temp.	Rate
10.45	15·5	30	3.0	30·0	66
11.55	15·0	30	3.2	30·0	*
12.30	0·2	3·2	3.5	30·0	Auricle stopped
12.40	0·2	3·2	3.15	20·5	40
12.55	4·8	8·8	3.20	20·5	40
1.5	4·8	8·8	3.40	12·0	19
2.5	20·0	38	3.50	12·0	19
2.55	20·0	37	5.20	13·0	22

* Partial heart block and irregular auricular beat.

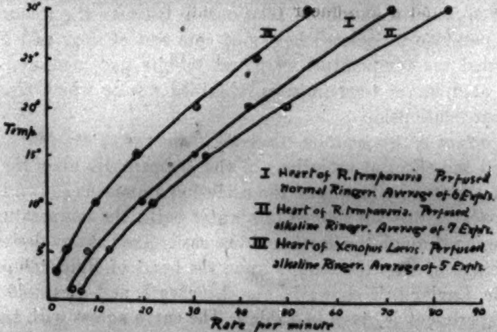

Fig. 2. The three curves show that the relation between temperature and frequency is altered when the perfusion fluid is modified, and also that the relation is different in different species of amphibia.

During these experiments with ordinary Ringer's fluid I noted that the frequency and force of the hearts diminished rapidly when the temperature was raised to 30° C., and even when supplied with oxygen, and since Weizsäcker(11) had shown that the oxygen consumption of the frog's heart increased rapidly when the temperature was raised, it appeared possible that the hearts, at high temperatures, were poisoned by accumulation of acid products of metabolism around the cells. I therefore tried the effect of increasing the buffer action of the Ringer's fluid by raising the concentration of the bicarbonate to 0·2 p.c. Unfortunately this change also involved a change in the reaction of the perfusion fluid, whenever oxygen was perfused through the inflowing fluid.

I found that when ordinary Ringer ($NaHCO_3$ 0·015 p.c.) was used and oxygen was bubbled through the fluid in the inflow cannula, the P_H of the fluid entering the heart was about 8·5, but that the P_H of the outflowing fluid was about 8·0, and therefore the P_H of the fluid in contact with the heart was very near 8·0. But when oxygen was passed through Ringer's fluid containing ·2 p.c. $NaHCO_3$, the P_H rose rapidly to between 9·0 and 9·5 and the P_H of the outflow was about 9·0; so that the P_H of the fluid in contact with the cells of the heart must have been over 9·0.

The Ringer's fluid with a $NaHCO_3$ content of 0·2 p.c. I shall term alkaline Ringer. When alkaline Ringer was substituted for normal Ringer the rate of the heart was slightly increased at normal and subnormal temperatures, but, when the temperature was raised above 20° C., then this change produced a marked increase in the rate and also the heart maintained the frequency and force of its contractions much better at temperatures above 30° C. when perfused with alkaline Ringer. Table II shows a sample protocol of one experiment in which the heart of Rana temp. was perfused with alkaline Ringer and the temperature varied and Fig. 2, curve II, shows the average result obtained from seven experiments.

TABLE II. R. temp. isolated heart perfused with alkaline Ringer.
($NaCl$ 0·45 %, KCl 0·014 %, $CaCl_2$ 0·012 %, $NaHCO_3$ 0·2 %, P_H – 9·0.)

Time	Temp.	Rate	Time	Temp	Rate
10.5	14	30	12.10	12·8	25
10.25	13·6	26	12.15	21·0	52
10.35	13·6	26	12.30	21·0	53
10.50	5·6	12	12.40	29·5	92
10.55	5·6	11·4	12.50	29·5	84
11.15	0·4	6	12.55	21·2	54
11.30	0·4	5·8	1.5	21·2	54
11.40	6·4	13·5	2.25	16·2	37
11.50	6·4	13	2.47	16·0	36
12.0	12·8	25·5			

A third series of experiments was made at Cape Town using the heart of a local toad, the Plaat Anna or Plathander (Xenopus lævis); this was perfused with alkaline Ringer. A sample protocol is shown in Table III and the average result of five experiments is shown in Fig. 2, curve III.

TABLE III. Xenopus lævis (Plaat Anna), isolated heart perfused with alkaline Ringer.

Time	Temp.	Rate	Height of ventricular contraction (movement of lever in mm.)	Vol. output per beat in c.c.
3.0	16·0	25	10·5	0·14
3.35	15·6	19	12·0	0·19
3.50	15·6	19	12·0	0·192
4.5	3·0	1·7	12·0	0·42
4.15	2·8	1·5	11·0	0·33
4.45	16·0	21	13·0	0·21
4.55	16·0	21	12·0	0·22
5.5	29·0	54	6·0	0·093
5.25	29·0	56	6·0	0·085
5.45	15·6	25	10·0	0·17
6.10	15·6	24	9·0	0·18
6.45	15·6	28	6·0	0·15

The three curves in Fig. 2 show firstly that, when one species of frog is used, the variations in frequency, observed when the temperature is altered, are different when the composition of the perfusion fluid is altered, and secondly that, when different species of amphibians are used, these variations in frequency are again different.

As regards the mathematical relations of these curves it is evident that not one of the three curves is a straight line, and I was unable to find any simple logarithmic formula that would express the relation between frequency and temperature; moreover the temperature coefficient for a rise of 10° C. is not a constant for any curve, and is different in each of the three curves. These coefficients are shown in Table IV.

TABLE IV. The coefficient of the increase of frequency of frog's hearts for a rise of temperature of 10° C. (Q_{10}). The frequency of the heart at x° C. $= Kx$.

	Perfusion fluid	$K10°/K0°$	$K15°/K5°$	$K20°/K10°$	$K25°/K15°$	$K30°/K20°$
1. R. temp.	Ringer	5·0	3·1	2·1	1·8	1·69
2. R. temp.	Alkaline R.	3·7	2·54	2·27	2·03	1·66
3. Plaat Anna	Alkaline R.	—	4·75	3·1	2·26	1·74

Since the relation between frequency and temperature is altered by varying the composition of the perfusion fluid and also is different for different species of amphibia, it appears to me to be of little use to attempt any exact mathematical analysis of this relation.

The peculiarities shown by the heart of the Plaat Anna in its response to variations of temperature are of interest. These animals live in water around Cape Town and are never exposed to frost, and in hot weather they get into shady pools where the temperature probably never rises above 28° C. The isolated heart of the Plaat Anna was much more sensitive to cold than the heart of an English frog, for the lower limit of the activity of the former was 3° C., at which temperature it quickly ceased to contract, whereas the heart of Rana temp. beat regularly at 0° C. and only ceased to beat between – 1° and – 2° C. The upper limit of activity was the same in both species namely 34° C., at which temperature the hearts ceased to beat after a few minutes.

The action of temperature upon the frequency of the isolated mammalian auricle. I found that when I perfused isolated mammalian hearts with Ringer's fluid it was extremely difficult to maintain a supply of oxygen sufficient to keep the heart beating with a regular frequency and force for more than about half an hour. I therefore employed instead of the whole heart the isolated auricles. Erlanger(2) first described a method of investigating isolated mammalian auricles suspended in Ringer's fluid, and the method I employed was similar to his. The auricles were cut off from the ventricles and were suspended by the appendages in a bath of Ringer, through which a rapid stream of oxygen was passed. The apparatus used is shown in Fig. 3. It was immersed in a water bath. I found that under these conditions the auricles beat regularly and strongly for many hours, in one experiment for instance, after 6 hours the rate was 80 p.c. and the height of contraction 85 p.c. of the figures recorded at the commencement of the experiment.

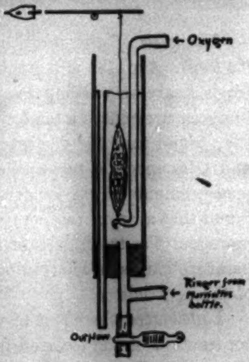

Fig. 3.

In order to maintain a regular beat it is essential to keep a strong current of oxygen passing through the fluid, any alteration in the oxygen supply immediately affects the activity of the auricle: even the addition of non-oxygenated Ringer to the fluid around the auricles affects their beat, and therefore it is necessary to oxygenate the supply of Ringer thoroughly, and also to maintain a regular flow of fluid, for it is very difficult to ensure that the fresh Ringer entering the bath around the auricles is as completely saturated with oxygen as is the fluid already in the bath, through which a rapid current has been passing.

With this apparatus I measured the effect upon the frequency and height of contraction of the isolated auricle of alterations of temperature, and the result of a typical experiment is shown in Fig. 4. This result resembles those obtained with the frog's heart, the frequency was not a linear function of the temperature, nor was it a simple logarithmic function; moreover the coefficient of the alteration of the frequency for a rise of 10° C. (Q_{10}) was not constant, the coefficient $\frac{\text{frequency at } 30^\circ \text{ C.}}{\text{frequency at } 20^\circ \text{ C.}}$ being 3·1 and that of $\frac{\text{frequency at } 40^\circ \text{ C.}}{\text{frequency at } 30^\circ \text{ C.}}$ being 2·2.

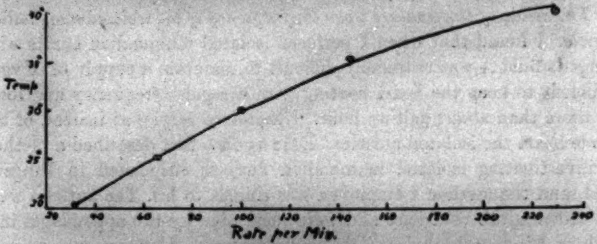

Fig. 4. Relation between temperature and frequency in the isolated auricle of the rabbit.

The effect of alterations of temperature upon the different functions of the heart. Amsler and Pick(1) took mechanical records of the isolated frog's heart at varying temperatures, and showed that an increase in temperature caused a marked diminution in the *a–v* interval; for instance they found that the *a–v* intervals at 17°, 25°, and 30° C. were 0·32, 0·11 and 0·05 seconds respectively. Seeman(7) took electrocardiographic measurements of the isolated frog's heart at different temperatures and found that a rise of temperature caused a shortening of the *P–R* interval; moreover the tracings that he has published show that a rise of temperature caused a shortening of the duration of the electrical response of the ventricle.

I measured the effect of variations of temperature upon the force of contraction of the isolated frog's heart and the rabbit's auricle. Fig. 5 shows the effect of variations of temperature upon the rate, the height of contraction and the volume output per beat of the heart of the Plaat Anna. Fig. 6 shows the effect of alterations of temperature upon the rate and force of contraction of the auricle of the rabbit.

With the help of Dr H. de B. Daly I made a series of experiments in which both the mechanical movements and the electrical movements of the frog's heart were recorded. Rana esculenta were used, and the methods employed for recording were nearly the same as those described by Mines(6). The effect of an increase of temperature upon the heart is shown in Tables V and VI. The alteration in temperature was obtained by altering the temperature of the fluid perfusing through the heart, but as it was necessary to expose the outside of the heart to the air, the temperatures recorded are only approximately correct.

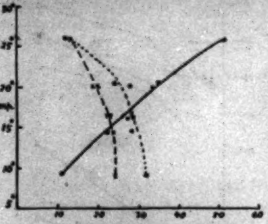

Fig. 5.

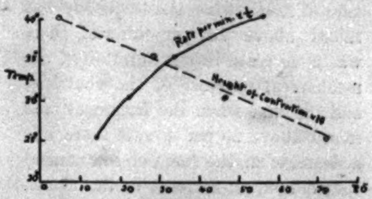

Fig. 6.

Fig. 5. Isolated heart of Xenopus lævis perfused with alkaline Ringer. —•— Rate per minute. - - -o- - - Height of ventricular contraction in mm. (movement of heart, × 20). – – × – – Volume output per beat, in cc., × 200.

Fig. 6. Effect of alteration of temperature upon the rate and the height of contraction (in mm.) of the isolated auricle of the rabbit.

Table V shows that an increase in temperature causes an increase in frequency, a decrease in the force of contraction, a decrease in the duration length of the electrical response of the ventricle, and a decrease in the length of the *a–v* interval. It is obvious however that these effects are of two kinds, firstly, the primary changes due to the rise of temperature, and secondly, the secondary changes due to the alteration in

TABLE V. Heart of R. esculenta perfused with normal Ringer.

Temp.	Length of cycle in secs.	Height of mechanical response of ventricle (movement of lever in mm.)	Duration of interval between *P* and *R* waves in secs.	Duration of electrical response of ventricle in secs.	Duration of mechanical response of ventricle in secs.
18	1·30	11	0·39	0·82	0·89
24	0·95	9	0·29	0·70	0·66

TABLE VI. Heart of R. temp. perfused with normal Ringer.

Temp.	Frequency	Length of *a–v* interval	Length of mechanical response of ventricle
13·5	26	—	1·3
15	29	0·5	1·2
18	37	0·38	1·2
21	46	0·30	0·9
24	58	0·22	0·86
30	72	—	0·6

frequency. In order to distinguish between these primary and secondary changes we made a series of experiments with hearts, to which a Stannius ligature had been applied, and which were stimulated at a constant rate by means of secondary induction shocks.

The effect of alterations of frequency upon the functions of the heart. A few experiments were first made to determine the effect of alterations of frequency at constant temperature. **Mines**(6) showed (i) that at a temperature of 12° C., when the frequency was raised above 10 per minute, there was a decrease in the duration of the mechanical response of the ventricle, and (ii) that when the frequency was raised above 20 per minute there was a decrease in the force of mechanical contraction, an increase in the length of the *P–R* interval, and an increase in the length of time taken for the rise of the *R* wave. He argued that the alteration in the last-mentioned measurement showed that an increase in frequency caused a diminution in the rate at which the wave of electrical excitation was propagated through the ventricle, and since an increase of frequency decreased the duration of the electrical response of the ventricle, therefore the increase in frequency must have diminished the length of the wave of electrical excitation. **Mines** concluded from these facts that an increase in frequency resulted in a shortened wave of electrical excitation travelling at a reduced speed through the ventricle.

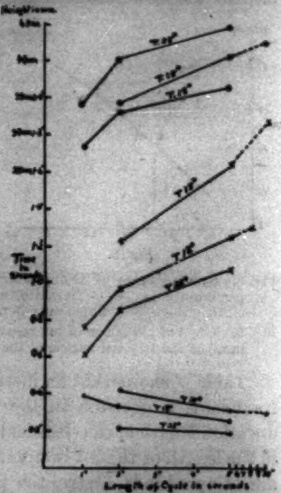

Fig. 7. Effect of alteration of frequency at three different temperatures on the heart of R. esculenta perfused with normal Ringer. —⊙— Height of contraction in mm. × 10. —×— Duration of mechanical response of ventricle in secs. —•— Duration of a–v interval in secs.

The results obtained by us with the heart of R. esculenta in summer agree with the results of **Mines**, who worked with R. temp. in winter. The effects on the movements of the frog's heart of alterations in frequency are shown in Fig. 7, and the effects upon the movements and on the electrical response are shown in Table VII. Our results agree exactly with the results obtained by **Mines** except that in some instances we found that a decrease in frequency below a certain figure caused a decrease in the force of contraction; this was probably due to the flow of fluid through the heart per minute becoming too small to oxygenate the heart sufficiently when the frequency fell below a certain figure. The galvanometer that we used did not unfortunately give records of the rate of rise of the *R* wave sufficiently accurate for reliable comparisons to be made.

TABLE VII. Heart of R. esculenta perfused with normal Ringer. A Stannius ligature was applied to the heart, which was stimulated with secondary induction shocks at varying frequencies. Temp. 21° C.

Length of cycle in secs.	Height of ventricular contraction in mm.	Duration of interval between *P* and *R* waves in secs.	Duration of electrical response of ventricle in secs.	Duration of mechanical response of ventricle in secs.
6·0	29	0·24	1·32	1·40
2·07	41	0·28	1·01	1·33
1·15	33	0·34	1·00	0·96

The effect of alterations of temperature when the frequency of the heart is kept constant. The effect of alterations of temperature, when the frequency is kept constant, upon the movements of the heart is shown in Fig. 8, and the effects of the same change upon the movements, and upon the electrical response, of the heart are shown in Table VIII; moreover a comparison of the different curves shown in Fig. 7 will show the

Fig. 8. Effect of alteration of temperature, when the frequency is kept constant upon the heart of R. esculenta perfused with normal Ringer.

TABLE VIII. Heart of R. esculenta perfused with normal Ringer. A Stannius ligature was applied to the heart, which was stimulated 29 times a minute with secondary induction shocks.

Temp.	Height of ventricular contraction in mm.	Duration of interval between *P* and *R* waves	Duration of electrical response of ventricle	Duration of mechanical response of ventricle
17	39	0·60 secs.	1·64 secs.	1·64 secs.
21	41	0·28	1·01	1·33
27	57	0·18	0·74	0·84

effect of differences of temperature upon the mechanical functions of the heart at three different frequencies.

These results show that when the frequency is kept constant an increase of temperature causes a slight increase in the force of contraction, a great decrease in the length of both the *P–R* and the *a–v* intervals, and a decrease in the duration of both the mechanical and the electrical response of the ventricle. A rise of temperature therefore causes an increase in the rate of conduction and an increase in the force of contraction, which are the opposite effects to those produced by an increase in frequency; but both a rise in temperature and an increase in frequency cause a diminution in the duration of the electrical response of the ventricle; but, whereas the increase of frequency was believed by Mines to produce this diminution in the duration of the ventricular response in consequence of a shortening of the length of the wave of electrical excitation, on the other hand, when the temperature is raised the increase in the rate of conduction may account for the diminution observed in the duration of the electrical response, and in this case there is no need to assume that the length of the wave of electrical excitation is altered.

The effect of alteration of temperature when the frequency is allowed to vary. The results shown in Tables VI and VII were obtained with intact hearts, which were allowed to vary spontaneously when the temperature was altered. The effects produced by a rise of temperature under these conditions were a decrease in the rate of conduction, a decrease in the force of contraction, and a decrease in the duration of the electrical and mechanical responses of the ventricle. These changes represent the algebraic sum of the primary effects produced by a rise of temperature, and the secondary effects due to the consequent increase in frequency. Table IX summarises the effects produced when the temperature and frequency change separately, and also those produced when they change together. This table shows that a rise of temperature produces an increase in the force of contraction, but an increase in frequency produces a greater decrease, and therefore when temperature and frequency increase simultaneously the result is a decrease in the force of contraction; a rise of temperature produces a great increase in the rate of conduction and an increase of frequency produces a smaller decrease in this rate, and therefore when both vary simultaneously the rate of conduction is increased.

The influence of a rise of temperature upon the metabolism of the frog's heart has been studied by Weizsäcker(11), who measured the oxygen consumption of the heart both at rest and when performing work: he showed that when the temperature was raised, and the frequency allowed to vary, the oxygen consumption increased very greatly, and that when the temperature was raised and the frequency kept constant, the oxygen consumption of the heart was again increased, although to a less extent than when the frequency was allowed to increase: his results also showed that when the temperature was raised and the frequency was allowed to vary, the oxygen consumption increased at a much greater rate than did the work done by the heart.

The primary effects of a rise of temperature upon the frog's heart are therefore (i) an increase in the frequency, (ii) an increase in the rate of conduction through the heart, (iii) an increase in the force of contraction, and (iv) an increase in the rate of oxygen consumption.

TABLE IX. Summary of the changes produced in the functions of the frog's heart by alterations in frequency and in temperature.

		Alteration in the height of the ventricular contraction	Alteration in the rate of conduction from auricle to ventricle	Alteration in the duration of the electrical response of the ventricle
I	Temp. constant. Frequency increased from 29 to 52 per minute	−20 %	− 21 %	−10 %
II	Frequency constant. Temp. increased from 17° to 21°	+ 5	+100	−36
III	Temp. increased from 18° to 24°. Frequency allowed to change spontaneously (frequency increased from 46 to 63 per minute)	−20	+ 25	−15

SUMMARY.

1. In both the frog and the rabbit, the frequency of the heart is not a linear function of the temperature, nor is it a simple logarithmic function.

2. The relation between the frequency of the heart and the temperature is different in different species of amphibia, moreover in the same species of frog, this relation alters when the hydrogen ion concentration of the perfusion fluid is altered.

3. The effect of a rise of temperature on the frog's heart, when the frequency is kept constant, is to cause an increased force of contraction, an increased rate of conduction from auricle to ventricle, and a diminution in the duration of the electrical response of the ventricle.

4. The effect of a rise of temperature upon the frog's heart when the frequency is allowed to vary is to cause a diminution in the force of contraction, an increase in the rate of conduction from auricle to ventricle, and a diminution in the duration of the electrical response of the ventricle.

5. The effect of a rise of temperature upon the isolated rabbit's auricle, when the frequency is allowed to vary, is to cause a diminution in the force of contraction.

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THE USE OF THERMIONIC VALVES WITH THE STRING GALVANOMETER.

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Experience with thermionic valves used with wireless apparatus led to the belief that small physiological currents which were not detectable by present methods might be amplified by means of these valves and recorded. A galvanometer capable of recording quick changes of potential with accuracy must of necessity be less sensitive than one which can only record slow changes of potential, as sensitivity has to be sacrificed to aperiodicity; it would be an advantage if quick changes of low potential could be magnified so that the relatively insensitive aperiodic galvanometer would be capable of recording them.

The research aimed at the amplification of both quick and slow changes of potential without distortion of the final curves due to the inertia of the magnifying circuit and the recording instrument. Preliminary experiments were made with transformers and balancing batteries in circuit with the valves but were abandoned owing to the error introduced by the periodicity of the apparatus. The circuit eventually used was one devised by Dr W. H. Eccles(1); it consists of a valve placed in one arm of a Wheatstone's Bridge and is practically aperiodic. Professor Starling pointed out the advantages of this method over the others we had tried and up to the present it has given the best results.

During the research Forbes and Thacher(2,3) published a preliminary communication on the use of the thermionic valve in magnifying the action currents of the nervous system and later a full report on the same subject. We encountered some of the difficulties which they described and therefore shall only briefly refer to them in this paper.

A comparison of the normal and magnified electrocardiograms was taken as a criterion of the aperiodicity of the circuit although it was recognised that magnification of the electrocardiogram *ipse se* would not lead to any useful information. Three valves in cascade were necessary to give a convenient amplification; the relative string excursions with only two valves have been shown in a preliminary communication(4).

The circuit. A diagram of this has been given in our preliminary communication. It consists of a valve placed in one arm of a Wheatstone's Bridge, the other arms having non-inductive resistances of approximately 30,000 ohms, the exact resistance being determined by the type of valve used. The valve acts as a resistance amplifier, changes of grid potential alter the resistance of the valve and therefore the P.D. across the output terminals. The input terminals are connected to the non-polarisable electrodes and the output to the string galvanometer switchboard which was of standard type supplied by the Cambridge and Paul Scientific Instrument Company.

The string galvanometer was of the Edelmann pattern with a Cambridge fibre case attached. The electromagnets took 12 volts, 4·3 ampères and the string magnification was 400, the period being less than ·02 sec. Marconi V. 24 and French valves were used.

The method we have adopted in balancing the bridge is to keep the filament and grid voltages constant and to vary the plate voltage until the resistance of the valve is such that no current flows through the string. When three valves in cascade were used we balanced each bridge successively commencing with the one connected to the string galvanometer. The details of each circuit are given below the curves. As accidents to the string were frequent in spite of the string shunts being used we connected a milliammeter by a double pole change-over switch to the output terminals; this facilitated the balancing and saved the string from breakage during the preliminary operations.

When the valves are connected to the galvanometer switchboard the potentiometer will not as a rule be found to have a wide enough range to compensate for the skin current and any small current flowing due to the bridges being slightly out of balance; this is especially liable to occur if the system is adjusted before connecting up the tissue to be examined to the input terminals. To overcome this difficulty we have added a potentiometer in circuit with one of the non-polarisable electrodes and a resistance for varying the filament brilliancy of No. 1 valve. The resistance of No. 1 valve will vary according to the resistance of the tissue in the grid circuit, *i.e.* the tissue connected to the input terminals, it is therefore advisable to place a resistance approximating to that of the tissue in the grid circuit before adjusting. The valves should be allowed to warm up for at least five minutes before attempting a balance.

Magnification of the Electrocardiogram.

In order to compare the amplification of the valve electrocardiogram with the normal we have taken both records with the same string tension. The large deflection resulting from the magnification rendered a high string tension necessary otherwise the record would not be small enough to be measured on the plate.

In all the figures small oscillations superimposed on the heart variations are evident.

A normal and amplified frog's electrocardiogram is seen in Fig. 1 *A*, *B*, the magnification is approximately twenty times and details of the circuit are given below the figure.

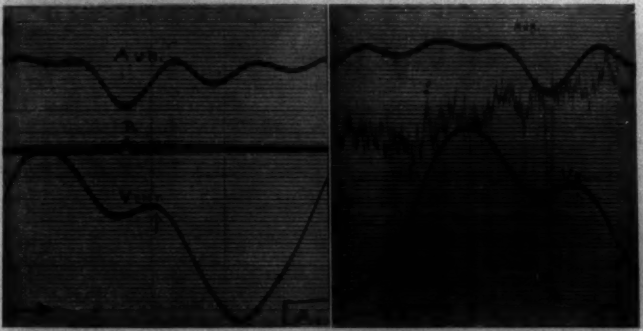

Fig. 1. *A*. Frog's electrocardiogram with mechanical record of auricle and ventricle. ·2 cm. × $\frac{3}{8}$ = 1 millivolt. *B*. Magnification with three V. 24 valves. Filament voltages, 5·8. Plate voltages of valves 1, 2 and 3 equal 42, 50 and 62 respectively. Light mains switched on. String tension same as in *A*. *B* is inverted in *B* owing to the grid input being placed on the wrong electrode.

The curves in Fig. 2 were taken to compare the form of the normal and amplified string excursions. The string tension in Fig. 2 *B* was increased in order to reduce the size of the deflection. The valve curves appear to be a faithful reproduction of the normal with the exception that the smaller oscillations are present.

The cause of the smaller oscillations on the magnified electrocardiograms. Forbes and Thacher found that electromagnetic waves from apparatus in the vicinity of the valves produced small string oscillations. We have met with the same trouble but do not find the valve Wheatstone's

Bridge circuit so sensitive to these disturbances as transformer and condenser circuits. Vibration and sound waves to a lesser extent affected the valves; the effect of the former has been almost completely nullified by mounting the valves on rubber. It was found that a very small disturbance of the galvanometer table would set up vibrations of the string undetectable in the ordinary way but very much magnified when the valves were connected. The magnification of these small string oscillations was due to the property of reactance of the valve circuit.

Fig. 2. *A.* Frog's normal electrocardiogram. *B.* Frog's electrocardiogram magnified by three V. 24 valves. String tension greater than in *A.* Light mains switched off.

The majority of the smaller string excursions seen in the curves appear to be caused by the variations of the D.C. in the lighting mains; the effect is seen in Fig. 1 *B* as compared with Fig. 2 *B*, when the electric light was switched off at the main. The variations in the D.C. supply were sufficient to cause a telephone connected to the valves to howl and on switching off the main light switch the howl disappeared. In Fig. 2 *B* the oscillations are relatively much smaller and are probably due to the heat and power circuits which had to remain on at the time the curve was taken; they were insufficient however to produce a howl in the telephones.

We believe that the circuit described above is of physiological value in that it may detect and magnify quick or slow changes of potential without distortion of the final curves, provided that the smaller oscillations due to extraneous electrical disturbances are avoided. With improved apparatus we hope to get rid of all outside electrical effects.

We wish to express our thanks to Dr W. H. Eccles for kindly interesting himself in the research and advising us on the circuit, also to Professor Bayliss for many helpful suggestions.

SUMMARY.

The use of thermionic valves with the string galvanometer in obtaining magnified electrical variations is discussed.

An aperiodic circuit devised by Dr Eccles consisting of a valve placed in one arm of Wheatstone's Bridge has been shown to give good results.

The difficulties met with in adjusting and maintaining a balance of the circuit, and avoiding oscillations due to outside electrical disturbances, are described.

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(2) **Forbes and Thacher.** Amer. Journ. of Physiol. 51, p. 177. 1920.
(3) **Forbes and Thacher.** Ibid. 52, p. 409. 1920.
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PHYSICAL EXERTION, FITNESS AND BREATHING.

BY HENRY BRIGGS, D.Sc., *Professor of Mining, Heriot-Watt College, Edinburgh.*

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THE experiments discussed in this paper were carried out during the research on mine rescue apparatus which was instituted in 1917 by the Scientific and Industrial Research Department[1]. Their aim at first was limited to that of determining the oxygen consumption of persons engaging in different kinds of physical work, and with that object in view, a few tests were made by Dr J. S. Haldane and the writer on miners climbing inclines in the Newbattle and Lingerwood collieries, Midlothian; these were shortly afterwards supplemented by other tests on the same men in which weights were lifted and certain of the common tasks of the miner were performed. During the early trials the men breathed ordinary air; but as the wearer of a mine rescue apparatus has to breathe air highly enriched with oxygen it was judged necessary to study the influence of such air on a person's capacity for physical work. It had been a matter of experience to the members of the research committee that they could perform work with greater ease and comfort while wearing a rescue apparatus in good order, and thus obtaining air containing 70 or 80 p.c. of oxygen, than under normal conditions. The writer, for example, can climb a mountain faster and with less fatigue when using an efficient mine rescue apparatus than without it, notwithstanding that the apparatus weighs, in its latest form, about 30 lbs. It was observed that an increased oxygen proportion in the air inhaled was uniformly helpful with persons of sedentary habits, but that when working miners were tested little or no such benefit was derived; they were in fact generally quite indifferent as to whether they breathed air or oxygen.

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[1] The research committee consists of Mr William Walker, C.B.E., H.M. Chief Inspector of Mines (Chairman), Dr J. S. Haldane, F.R.S. and the writer. Summaries of these experiments have been published in the writer's paper on "Fitness and Breathing during Exertion," this *Journal*, 53, *Proc Physiol. Soc.* p. xxxviii, 1919, and in the *Second Report* of the Mine Rescue Apparatus Research Committee, 1920.

A long series of experiments was then commenced in which Martin's ergometer was principally used as the means of measuring the rate of exertion, and in which a quantitative and qualitative examination was made with subjects of various physique and training breathing air and oxygen. Thanks to the kindness of the Superintendents of Physical and Bayonet Training, Scottish Command and Aldershot, several soldiers specialising in different branches of athletics were included in the tests.

Subsequently, when it had become clear that the fitness of a subject could be measured by contrasting his respiratory performance when breathing normal air, and when breathing enriched air, the Army Council, acting upon the recommendation of Colonel Sir William Horrocks, K.C.B., and Lt.-Colonel E. P. Cathcart of the Army Medical Department, set up a Physical Test Station at which, up to the Armistice, the new method was applied for the examination of men sent in from units under the Scottish Command.

APPARATUS AND METHODS.

The ergometer experiments were carried out in the Heriot-Watt College, Edinburgh, with *Martin's ergometer* (1). During the experiments a pendulum, hanging in front of the subject as he sat in the saddle, provided the means of timing the rate of revolution of the pedals[1]. A rate of 56 revs. per min. was adopted throughout. At that speed, and with the gear-ratio of the particular cycle employed, the power expended, in foot-pounds per min., was ascertained by multiplying the difference of the balance-readings, in pounds, by one-thousand.

Meters. Two Milne dry meters were used. In each of them one revolution of the 8 ins. pointer indicated the passage of one cubic foot of gas. The dials were marked off in hundredths of a cubic foot. The meters were tested against displacement from time to time. The barometric pressure, temperature and hygrometric state of the gas being metered were kept under observation.

Douglas bag and sampling apparatus. A 60 litre wedge-shaped bag was used to collect expired air. The bag, which is part of the Douglas respiration apparatus (2), is provided with a 3-way aluminium stop-cock which allows of the expired air being either discharged direct to the atmosphere ("off" position) or into the bag ("on" position). A small rubber tube connected to the bag enables samples of the contents to be drawn off for analysis. At first glass sampling tubes with taps at each end were used for this purpose, but they were soon abandoned in favour of small well-stoppered bottles, filled over a mercury trough. In the large numbers required the bottles were handier, simpler to use, less costly and much more easily replaced in case of breakage. A further simplification in taking samples for analysis was introduced at the Test Station.

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[1] For long-continued pedalling the pendulum is preferable to the metronome for this purpose; it is less trying to the nerves.

Oxygen cylinders and reservoir bag. When the subject was breathing oxygen the gas was supplied from a 100 ft. cylinder fitted with a reducing valve. The oxygen discharged from the valve into a reservoir bag. At first the subjects complained of parching of the throat when breathing oxygen; the trouble was removed by causing the gas to bubble through water before entering the reservoir. The latter was kept about three parts distended during a "run." Over-distension was carefully avoided, as with excess of pressure in the reservoir bag it became possible for the gas to push open both breathing valves and to discharge direct into the Douglas bag.

Mouthpiece, valves and tubes. The subject used a rescue-apparatus mouthpiece of rubber which fitted over one limb of a metal T-piece. The nose was closed by a clip. The air was directed to and from the mouthpiece by inspiratory and expiratory valves. Various valves were tried, the most successful being the Mueller water valve (Fig. 1) and the Rosling valve (Fig. 2). A Mueller valve of the dimensions indicated in Fig. 1 allows of the heavy breathing of severe exertion without introducing a degree of resistance appreciable to most persons.

The Rosling valve, adopted towards the end of the war for Army anti-gas purposes, is very free from resistance and low in slip. While Mueller's is only serviceable for a stationary subject in the laboratory, Rosling's is equally useful in the laboratory and in testing men marching or climbing in the open or in the mine. Unlike the mica disc valve so frequently used in respiration experiments, it functions properly in any position. The valve is of rubber. A thin square of rubber, held by the corners, closes upon the flanged end of the tubular part of the valve (Fig. 2, left-hand view) and flexes away from it when allowing air to pass through (Fig. 2, right-hand view). The valve here illustrated was made for the writer by the Isleworth Rubber Company and adopted in the Briggs Mine Rescue Apparatus. Its resistance to a flow of 85 litres (3 c.ft.) of air per minute is 0·35 in. of water column, and its slip is quite negligible. A not unimportant feature of the rubber valve is its noiselessness. When a valve makes a distinctive noise the subject's attention is apt to be directed to his own breathing, and the test may be vitiated thereby. The tubes leading to and from the mouthpiece were of one inch bore,—a size sufficient to reduce their resistance to negligible magnitude even with hard panting.

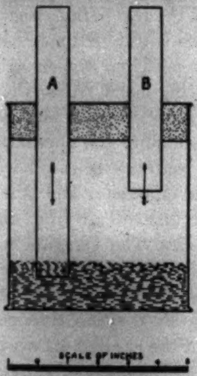

Fig. 1. Mueller Water Valve.

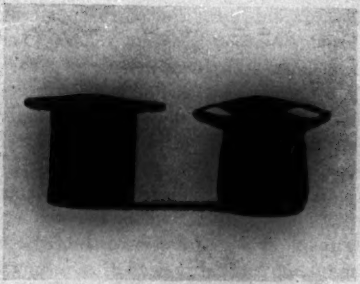

Fig. 2. Rosling Valve, shut and open.

Sampling tube for alveolar air. A number of samples of alveolar air were taken while certain of the subjects were pedalling the ergometer. Essentially, the apparatus used for this purpose was that described by Haldane and Priestley(3); it consists of a long tube through which the subject can empty his lungs suddenly. To avoid the subject having to close the end of the tube with his tongue after such an expiration, as was done in Haldane's and Priestley's experiments, a wide-bore tap was fitted at that end; on this being closed the man could replace the rubber mouthpiece (which for the moment he had withdrawn) and continue working, leaving the experimenter to draw out from behind the tap a small sample of air for analysis. With the quickened breathing incident to physical work, it was not possible to get alveolar air samples after inspiration and after expiration as may be done under rest conditions. Most of the experiments were on men altogether strange to scientific methods; few were sufficiently trustworthy when it came to the difficult operation of providing a reliable alveolar sample while doing hard work.

Gas-analysis apparatus. In the main set of experiments, in which both oxygen and carbon dioxide were determined in each sample of expired air, the Haldane gas-analysis apparatus(4) was used. At the Test Station, where the routine was simplified and only CO_2 ascertained, the Briggs' apparatus(5) was adopted.

Manner of conducting experiments. A number of preliminary trials were carried out in order to settle such matters as the rest period needed between spells of work on the ergometer, and the length of time work must continue before the breathing becomes sufficiently *en rapport* with the exertion to permit of reliable observations being made. After that, a regular routine, based on Douglas' method, soon evolved, and the few changes subsequently made were merely to simplify the apparatus and connections and to improve the valves. This routine was as follows: The subject, seated at rest on the saddle of the ergometer and fitted with the noseclip and mouthpiece attachment, inhaled air from the room, drawing it through one of the Milne meters. The Douglas bag (now empty) was connected to the exhalation tube with the 3-way cock in the "off" position to allow the products of respiration to escape into the room. When he had become accustomed to his position the cock was turned "on" at the end of an inspiration and the expired air began to enter the bag. A stop watch was started at the moment of turning the cock, and the number of inspirations was counted by watching the movement of the pointer of the meter. After about two minutes had elapsed, and again at the end of an inspiration, the tap was turned "off," and the watch stopped. The bag was kneaded and one or more samples drawn from it. The volume in the bag was then measured by emptying the bag through the second meter. The necessary thermometer and hygrometer readings were taken. From these measurements and the time interval the volume exhaled per minute was calculated. The meter on the inspiration side enabled the quantity drawn into the lungs to be evaluated direct. Owing to the jerky action of the latter meter this determination did not reach the same degree of accuracy as that of the volume of exhaled air from the Douglas bag, but it was useful as a safeguard against gross error.

The second set of readings and samples were taken when the subject was pedalling with the belt off, *i.e.* when he was doing no external work. The same routine was followed, the man being required to pedal at the rate of 56 revs. per min. for at least two minutes before commencing to collect the expired air. After this, similar records were obtained with gradually increasing loads. Longer rest intervals were allowed between the spells of work as the loads increased. The preliminary interval of two minutes' pedalling was strictly observed, except for the highest loads, such as 12,000 or 14,000 ft. lbs. per min., which are beyond the capacity of even the strongest men to sustain for long. With excessive loads the preliminary interval had perforce to be shortened, though it was never allowed to be under one minute. The reduction of that interval, however, makes the determination of oxygen-consumption, etc., on the highest loads less reliable than those on more moderate rates of work, a feature which receives further consideration below.

After making a series of measurements with the man breathing air, an exactly similar series was made when breathing oxygen. Before commencing the latter the subject was required to breathe oxygen for at least ten minutes to expel the greater part of the nitrogen dissolved in the blood. The interval between the air and oxygen tests was usually some hours; on several occasions the two series were carried out on different days. In the cases of several of those tested at the College, and of nearly all those tried at the Test Station, no information was given to the subject as to whether he was breathing oxygen or air. It was thought best not to give a loophole for the prejudice, which is still curiously strong, against breathing oxygen for a few hours.

After a few tests with a simple pneumograph it was abandoned. As has been stated, the rate of breathing was ascertained by watching the pointer of the meter on the inspiration side, and as the meter had its back to the subject he was generally unaware that any notice was being taken of his breathing.

ERGOMETER TESTS.

Figs. 3 to 14 (see end of this Paper) are typical graphed records of subjects undertaking work on the ergometer in the manner described. The small circles on the charts indicate values obtained when the men were breathing normal air and the crosses those obtained when breathing oxygen; the more-or-less smoothed "air" curves are drawn in full lines and the "oxygen" curves in dotted lines. The output of CO_2 and the oxygen consumption are set out in litres per minute of dry gas at N.T.P. The volume ventilating the lungs is given in litres of saturated air or oxygen at blood temperature and normal pressure, expired per minute. The oxygen consumed was computed from the respiratory quotient and the CO_2 output.

The determination of the R.Q. by gas-analysis depends on the assumption that the mass of nitrogen inhaled is the same as the mass of nitrogen exhaled. The nitrogen, in fact, serves here as a measure or standard against which variations in oxygen are gauged. Evidently such a process will be more accurate when the nitrogen, as in ordinary air, exceeds the oxygen in volume: *i.e.* when the smaller is gauged by the greater. It will be less accurate when the nitrogen proportion is much lower than that of oxygen, as when cylinder oxygen is breathed, for then the greater is gauged by the lesser. As might therefore be expected, oxygen consumptions, calculated in the manner indicated, from measurements made when breathing cylinder oxygen, had a relatively high probable error. Another method of evaluating oxygen consumption was, however, applicable, owing to the volumes inhaled and exhaled being separately measured; and in case of doubt this second method was used as a check. It consists of finding (*a*) the volume of oxygen entering the lungs per minute (from the volume of enriched air inhaled and the proportion of oxygen in that inhaled gas); (*b*) the volume of oxygen leaving the lungs per minute (from the volume expired per minute and the proportion of oxygen in the expired air), when the difference (*a*)—(*b*) gives the required result.

Towards the end of the main series of experiments it was found that, even in a subject of low fitness, no advantage was to be secured by increasing the percentage of oxygen above 60. Had that fact been known earlier, the work would have been facilitated by using a mixture containing 60 p.c. oxygen and 40 p.c. nitrogen in place of cylinder oxygen; the higher proportion of nitrogen which would then have been available would have reduced the probable error of the oxygen consumption determinations on enriched air. At the Physical Test Station 67 p.c. of oxygen was used instead of cylinder oxygen.

It has sometimes been advanced as a drawback to the Douglas method that the sample of exhaled air collected in the bag is obtained over too short a period. For work of the character now being considered, however, the criticism would not appear to have much weight. If elementary precautions are taken, such as that of opening and closing the bag at the same stage in the breathing (*e.g.* at the end of inspiration), the shortness of the period of collecting the expired air is not a matter of consequence; of much greater importance is the length of the preliminary period during which the man is required to work before the expired air is allowed to enter the bag. This should be uniform and adequate.

It was not practicable to put the subjects on a definite dietary. While this increased the degree of uncertainty of any single pair of observations it does not affect the general results, since a large number of men were tested, and whenever doubt was felt in regard to the reliability of a set of measurements the test was repeated on another day.

Normal and overload. Every-day experience proves that a muscular performance is easier when one is in "good condition." Equally commonplace are the facts that no task involving external work, not even the lightest, can be continued indefinitely without pause, and that the heavier the work the shorter the time it can be sustained. There are, however, certain lesser degrees of exertion (for instance walking or cycling at a moderate pace on a flat road) which, by the ease with which they can be kept up for hours on end, may be referred to, in electrical engineers' phraseology as "normal loads"; while other and heavier tasks (*e.g.* hard bayonet exercise or running quickly upstairs) are bearable for a limited period only and may be termed "overloads." What may be an overload to one person is a normal load to another who is stronger, or who is in better training or more habituated to the particular kind of labour. Again, a normal load when a person is fit may prove to be an overload when he is unfit; and, as has been remarked, even a light normal load if long supported without rest will eventually become an overload. Evidently, then, the whereabouts of the line demarcating between a normal and an overload for any individual depends on his condition at the time; if he is getting tired, it is moving down the scale of exertion; if resting, it is moving up.

Oxygen supply and carbon dioxide output during work. An important difference between what we here term the normal load and the overload lies in their effect on the respiration after stopping the exertion. When one ceases an easy normal load like walking at 3 m.p.h. along a flat road, the breathing quickly adjusts itself to the resting state: the after-effect in a healthy person is *nil.* The influence of a severe overload is in marked contrast to this; when the work is stopped heavy breathing continues; the lung-ventilation falls to normal only after a period which, in the case of a hard spell of work, may be many hours. In the first instance the oxygen intake was adequate; in the second it was not. Essentially, then, a normal load may be defined as one during the performance of which the oxygen supply is sufficient, and an overload one during which it is insufficient to satisfy in full the demands of the working muscles.

It is obvious that the supply of oxygen to the tissues may be deficient either in consequence of insufficient absorption in the lungs or inadequate circulation. Instances in which distress is produced by the rate of oxygenation of the blood failing to keep pace with the muscular demands, though the circulation may be sufficient, are of great practical interest. They include the case of the poison-gas patient, where exudation and thickening of the epithelial layer of the lungs make oxygen-penetration difficult; the case of the high-flying airman, where the low partial pressure of oxygen prevents proper oxygenation of the blood, and that of the so-called D.A.H. patient, where the shallowness of the breathing impairs the transfer of oxygen to the blood by insufficient exposure of epithelial area to freshly indrawn air (6).

A glance at the accompanying charts will show that when hard muscular work is being done the consumption of oxygen may rise to more than ten times the resting value. In the muscles at work there must be a much greater proportional increase of consumption, and such an increase can only be secured by an enormous addition to the blood circulation through those muscles. Failure to supply the additional blood, whether due to defects in blood-distribution or to cardiac efficiency, must, therefore, bring about local anoxæmia in the muscles, resulting in a cessation or reduction of the exertion.

Now it is known that, when muscles are insufficiently supplied with oxygen, lactic acid is formed; indeed that when an extreme overload is attempted, such as running quickly up several flights of stairs, the blood is at once flooded with lactic acid. The highly stimulative influence of lactic acid upon the respiratory centre and the relatively slow rate at which it disappears from the blood are also well known. The formation of this acid would therefore appear sufficient to account for the falling off of the percentage of CO_2 in the expired air which (as the curves show) is the invariable rule when the load is increased beyond a certain amount, and would also partly explain the long-continued enhanced breathing after the cessation of a heavy overload.

Since the appearance of lactic acid in the blood is a sure sign of overload, and since that appearance is characterised by a fall in the proportion of CO_2 expired, the writer feels justified in taking the rate of work corresponding to the maximal CO_2 proportion in the expired air as the boundary between an overload and a normal load, while breathing air or oxygen as the case may be. This boundary, it will be understood, can only be a rough one. Nor is it a stable one; fatigue moves it down the scale. Again, the fact that in most cases (*e.g.* Fig. 4) the expired-CO_2-percentage curve gradually flattens as the crest of the dome is approached, appears to denote the onset of oxygen-want before the "crest-load" is reached; but since that influence (except in the immediate region of the crest load) is not usually serious, the subject can support such rates of exertion for a considerable time. In other words, though there is in the charts good evidence that the call for oxygen by the working muscles becomes (either *per se* or through the agency of lactic acid) a partner in respiratory control even on relatively light exertion, the demand appears to be satisfactorily met until the rate of work is increased up to, or nearly up to, what is here termed the "crest load."

Stamina. Stamina is taken to mean the power of supporting continuous exertion. It will be apparent that the higher the "crest load" the larger will be the range of loads which can be dealt with without oxygen-want bringing the exercise to an end. A given rate of work may be a normal load to one man whose "crest load" is high and an overload to another whose "crest load" is low. Thus the crest load (the abscissal position of the crest of the dome of the exhaled-CO_2-percentage curve) becomes a measure of the stamina for the particular kind of work in question.

In every case but one (Subject VIII) the crest load was higher (*i.e.* the crest was further to the right) when breathing oxygen than when breathing air. In most cases, that is to say, the boundary between normal and overload moves up the scale, and the subject's capacity for sustained exertion is improved, as the partial pressure of oxygen in the inhaled air, and, therefore, in the alveolar air, is increased. The lower the person's fitness the greater the improvement brought about.

Alveolar CO_2 during the accelerative period. As has already been stated, a rate of work like 12,000 ft. lbs. per min. was too heavy to be kept up long by any of the men tested, and on such loads it was not possible to wait the usual two minutes before taking the samples and readings. The result was that on the heaviest loads, the latter were taken during the accelerative period. In some instances (Subjects II, III, IX, XIII, XV) alveolar samples were obtained during that period, and the CO_2-percentages are shown on the graphs. They will be seen to be unusually high; indeed with Subject XIII, breathing air, the record figure of 10·1 p.c. is reached. The matter lay outside the scope of the research and was not pursued further; but it would seem questionable whether these high CO_2 tensions are possible in the alveolar air without active excretion of CO_2 on the part of the lung-epithelium.

Fitness and expired-CO_2-percentage. The graphs may now be examined with a view to ascertaining the influence of fitness on respiratory behaviour.

The high level of the CO_2-percentage in the air expired by most fit persons doing work is perhaps the first feature to attract attention. It is a usual but not an invariable attribute of the fit man that he can stand a higher CO_2 and a lower oxygen percentage in the alveolar air than the unfit man performing the same task; he makes more use of the air he inhales and therefore requires less of it. Thus, in contrasting the very striking athletic subject XIII (condition (A), Fig. 10) with the sedentary subject II (Fig. 4) when both are breathing normal air, the maximal CO_2 percentage in the former case is seen to be 8·1 and in the latter case 4·7, and, although the heavier man, XIII can work the ergometer on a lung-ventilation of less than half that required by II. The highest CO_2 and lowest oxygen proportions were recorded in the case of those to whom slow, deep breathing is habitual during physical exercise. While working at the rate of 6000 ft. lbs. per min., for example, II breathed 24 times per min., while two unusually deep breathers, Nos. XIII and VIII, respired 8 times and 12 times per min. respectively on that load. That the correspondence of high fitness and a high CO_2 level is not invariable is shown by a subject (graphs not reproduced), a very fit young athlete, whose expired-CO_2-percentage reached a maximum of only 4·8 when breathing oxygen.

Fitness measurement. Perhaps the most interesting and useful of the results obtained follows from a comparison of the curves of exhaled-CO_2-percentage when the subject breathes air and when the subject breathes oxygen. In the case of a relatively unfit man, such as II, these curves diverge; but in that of VIII (Fig. 8)—an army instructor in physical drill selected for experiment by the Scottish Command as representing physically the best the Army can produce—the curves are almost coincident and their crests actually coincide. Observations on many subjects have warranted the conclusion that fitness is inversely as the degree of divergence of the two CO_2 curves. The most convenient manner of evaluating fitness proved to be the following: having drawn the two contrasting curves (work done, abscissæ; CO_2 percentages, ordinates) the expired-CO_2-percentage, with the subject at rest and breathing normal air, was marked by an arrow-head on the Y-axis of the graph. A horizontal line having then been struck across the chart through the arrow-head, the vertical distances between that line and the crests of the "air" and "oxygen" curves were measured off. The fitness factor was then taken to be the first of these distances divided by the second. By this method the fitness of Subject II was 46 p.c. and that of VIII was 100 p.c. The factors for the other selected subjects are stated in the Appendix (p. 311).

The assumption underlying this mode of expressing fitness is twofold: first, there is, as basis, the conception of zero fitness as being the state in which the CO_2-curve on air falls away from the Y-axis, or, in other words, in which the crest lies on that axis at a point coincident with the resting value of the CO_2-percentage. That is to say, zero fitness is regarded as the condition in which the slightest load is an overload and where oxygen want becomes serious when the least exertion is attempted. Secondly, there is the assumption that breathing oxygen raises fitness (as regards the lungs) to 100 p.c. The first point will be readily conceded; as to the second, the evidence appears conclusive. Subject III was tested on occasions several months apart; the first time he was in low health and his fitness factor was 44 p.c.; the second time he was well and the factor had risen to 80 p.c.; but the CO_2 curve on oxygen was substantially the same in each case. Subject XIII was frequently tested over a period of six months. At first he was in normal health and had a fitness of 70 p.c. He was then sent to Aldershot for the final course of training for sergeant-instructors in physical drill and returned to Edinburgh, in the "pink" of condition, for further test after being a fortnight at Aldershot. It was then found that while the "oxygen" curve was substantially as before, the "air" curve had risen to meet it, and that, indeed, the two curves agreed up to the crest. In other words, fitness had become 100 p.c. Some time after, XIII was transferred back to Scotland under medical orders; he had become very "stale" and run down. He was again tested and found to have a fitness of 55 p.c.; but, as before, the change was evidenced by a movement of the "air" curve only.

It is to be observed from the results that, when an overload is being dealt with, even the fittest men derive some assistance from breathing enriched air, while the unfit benefit to a still greater extent. An overload to a relatively unfit person breathing ordinary air may become a normal load when he breathes, say, 70 p.c. oxygen. A man getting fatigued while supporting what was at first a normal load but which has now become an overload, no matter how fit he may be, is relieved by breathing enriched air,—an effect which has been remarked by other observers. Conversely, heavy work can be accomplished with less fatigue when respiring oxygenated air *continuously* from the commencement.

The method of measuring fitness described above involves the assumption that lung-fitness indicates general physical fitness. Such appears actually to be the case if an exception be allowed in the instance of persons inured to living at a high altitude; in those circumstances the required degree of adaptation is not derived so much from physical exercise as from long-continued exposure to low oxygen pressure, and the lungs may be highly efficient without general bodily fitness being a necessary consequence.

Bearing on the oxygen secretion question. Since an unfit man derives much benefit during muscular exertion through addition of oxygen to the inspired air, while a fit man is very little benefited, it seems clear that the lungs of the fit man absorb oxygen more readily from normal alveolar air during exertion. This might be due either to some anatomical change which makes simple diffusion occur more readily through the lung epithelium of the fit man, or to active secretion of oxygen inwards by the lung epithelium.

The former theory does not seem inherently probable; but if it were correct we might expect that even during rest the alveolar CO_2-percentage would be higher among fit than among unfit men. To ascertain whether this is so the records of 84 men were examined. They were of every medical category, though the "A" class preponderated. Their ages ranged from 15 to 50, though most were of the usual military age. The following table sets forth the expired-CO_2-percentage sitting at rest against the fitness factor, the latter having been determined as described above:

TABLE I.

Fitness p.c.	Number of subjects examined	Average expired-CO_2-p.c. at rest
40–50	6	3·52
50–60	10	3·75
60–70	23	3·66
70–80	19	3·61
80–90	20	3·52
90–100	6	3·60

The evidence is emphatically negative; the expired-CO_2-percentage at rest, and therefore, by inference the oxygen tension of the alveolar air at rest, is not affected by a very large variation in fitness.

The secretion theory as propounded by Bohr and by Haldane and his co-workers affords a more probable explanation. The theory predicates that the epithelial cells possess the power, which they exercise in response to stimuli originating in anoxæmia of the tissues, of secreting oxygen from the alveolar air into the blood (7). When a person is at rest he gets oxygen by simple diffusion; but during work, or during existence at a high altitude, the amount so obtained is inadequate and is supplemented, as shown by the experimental data of these observers, by secretion.

Once these cells are regarded, so to speak, as oxygen pumps which can be set going when required, the experimental results described above become intelligible. Practice or training facilitates the oxygenation of the blood by improving the cells' power of secretion. In the fittest men, no benefit is derived during normal load from breathing enriched air, since they are able to get from normal air by secretion all the oxygen they need. The existence, in the lung epithelium, of a capacity which can be developed and intensified by training or other means of adaptation and which inferentially may be impaired by overwork or overstrain, throws a new light on the phenomena of respiratory fatigue.

Oxygen consumption. Table II, which has been drawn up from the smoothed curves, gives, in litres per minute of dry gas at N.P.T., the oxygen consumption of the selected subjects while doing work on the ergometer and while breathing both normal air and oxygen.

TABLE II. Oxygen Consumption. Ergometer experiments.

	Sitting at rest		Work done in foot-pounds per minute					
			3000		6000		9000	
Subject	Breathing air	Breathing O_2	Breathing air	Breathing O_2	Breathing air	Breathing O_2	Breathing air	Breathing O_2
I	0·28	0·31	1·23	1·05	1·77	1·55	2·42	2·15
II	0·23	0·31	1·23	1·20	2·08	1·82	2·42	2·30
III	0·37	0·22	1·03	0·81	2·62	1·30	2·20	2·10
IV	0·33	0·28	1·12	0·95	1·68	1·61	2·04	2·30
V	0·28	0·37	1·17	0·81	1·80	1·44	—	—
VI	0·47	0·40	1·17	1·02	—	—	—	—
VII	0·45	0·40	1·13	1·05	1·70	1·49	2·37	1·90
VIII	0·42	0·32	1·05	0·97	1·58	1·50	2·27	2·00
IX	0·40	0·30	1·00	1·05	1·68	1·62	2·30	2·30
X	0·37	0·28	1·12	0·81	1·83	1·28	2·52	1·70
XII	0·40	0·41	1·07	0·92	1·58	1·54	2·09	2·02
XIII (*a*)	0·33	0·45	0·70	1·05	1·17	1·54	1·75	2·20
(*b*)	0·37		1·30		2·00		2·65	
XIV	0·38	0·25	1·17	0·92	2·09	1·65	2·62	2·52
XV	0·25	0·35	1·07	0·98	1·63	1·50	2·30	2·30
XVIII	0·42	0·24	1·30	1·00	2·00	1·67	2·71	2·31
Average	0·36	0·32	1·12	0·97	1·74	1·54	2·33	2·16

Efficiency of Ergometer Work. The curves relating to efficiency at different loads have a certain interest, though of all the results these are perhaps most open to criticism and require most qualification. They were computed from the oxygen consumptions and from Zuntz's table of energy-equivalents (8). They are "gross" or "overall" efficiencies and give, at different loads, the relation between the useful external work done by the human machine and the energy generated within that machine by exothermic chemical changes. A person pedalling the ergometer with the belt off and thus doing no external work has, on this basis, zero efficiency. The accuracy of these estimates depends on several factors. The manner of arriving at Zuntz's figures has, the writer believes, been considerably criticised, though in view of the other sources of error now to be noted small imperfections in those values are barely worth considering. The evaluation of efficiency on normal load is comparatively straightforward and probably fairly exact, but grave difficulties arise when overloads are being dealt with. Being determined from the oxygen consumption, an efficiency is evidently affected by error in measuring the oxygen consumed. Further it is important to realise that, as a statement of the rate of oxygen consumption *at a given time*, say two minutes after starting an overload, a certain value may be accurate and yet it may yield altogether misleading results if used as the basis for calculating efficiency *at the same time*. This conclusion follows from the fact that to measure efficiency accurately there must be a correct correlation of energy-intake and energy-output. There is, however, no such agreement during an overload when the output of energy is, for a time, excessive. The portions of the curves which are considered unreliable for this reason are shown by even dots.

If efficiency had been the subject under study, it would have been necessary to endeavour to correct the curves by taking into account the excess oxygen consumption during the post-work period. An interesting feature (which has been noted by previous workers) becomes apparent when an efficiency curve is corrected in that manner; it is then seen to be dome-shaped: in other words, as the load increases the efficiency reaches a maximum and then falls away again. The maximum occurs at or near the line of demarcation of normal load and overload. Even with the uncorrected efficiencies graphed, the tendency towards the domed form can be detected in several cases, as for example in that of Subject IX. Zuntz's values being based upon the respiratory quotient, the abnormality of the R.Q. during severe work is another disturbing factor.

Generally speaking, the efficiency appears to be greater when breathing oxygen than when breathing air. With the relatively unfit person that effect may be partly due to the fact that, for a given load, less energy is consumed in respiration when breathing oxygen.

The efficiency of the fit is greater than that of the unfit man. This again may to some extent be owing to the relatively small respiratory effort of the fit person; but no doubt the fact of the fit man being usually habituated to physical exertion, and having learnt to deal with a task with a minimum waste of muscular energy as possible, has a great deal to do with his higher efficiency. For example, the expenditure of energy and consumption of oxygen involved when a miner uses a shovel are markedly less than when the same task is performed by a person unaccustomed to shovelling.

Climbing and Walking Experiments.

A number of experiments were made on men climbing the main incline of the Burdiehouse limestone mine, Midlothian, both while breathing normal air and while breathing oxygen. Preliminary tests in the Lingerwood and Newbattle Collieries had shown the advisability of limiting the variables. This could be done either by taking one subject on a number of gradients or by taking several subjects on one gradient, and the latter alternative was chosen as being likely to give most information. The Burdiehouse incline lies at a uniform slope of 21°. The roof is high, so that there was no occasion to stoop, and the floor, while dry for the most part, was, at the time of the tests, wet and slippery in places. On the whole the condition of the incline might be taken as a fair average of that of a mine roadway of heavy grade. Owing to the difficulties encountered in fitting up, each day, a temporary laboratory on the side of the roadway, I had to be satisfied with a few determinations for each man; usually values were obtained at five rates of speed, both when breathing air and when breathing oxygen.

It was intended to put the results, especially as to oxygen consumption, in the most useful form for designers and users of mine rescue apparatus; therefore the subject carried such an apparatus both during the climbing tests and during the walking and running trials on the flat which are referred to below. The total weight borne on each occasion was about 43 lbs. The values thus apply to fully-equipped infantrymen. The procedure during these experiments was the following:

Breathing normal air. The subject carried a Douglas bag on his back and an exhalation bag, fitted with a relief valve, on his chest. He breathed through a mouthpiece, his nose being clipped. Inhalation and exhalation valves were so placed that he drew air from the Douglas bag and expired into the exhalation bag. Before starting, the Douglas bag was inflated with a measured volume of air by aid of a large double-acting bellows. The man was then set to walk up the incline (which was marked off in chains and poles) at the desired rate. The 3-way tap of the Douglas bag being "off," he inspired, at first, from the atmosphere.

When it was judged that his respiration had adjusted itself to the degree of exertion, the 3-way tap was turned "on" and he began to breathe from the measured volume in the Douglas bag. The length of the spell of work, from the moment of turning on the tap to the moment of turning it off again, was taken by a stop-watch. After the spell samples for analysis were withdrawn, over mercury, from the exhalation bag, and the volume remaining in the Douglas bag was metered.

Breathing oxygen. Before any observations were made on oxygen, the man was required to use the mine rescue apparatus which he was carrying for a sufficient time to remove the bulk of the free nitrogen dissolved in the blood and tissues. During this preliminary period the nitrogen percentage in the air of the closed circuit of the apparatus was kept low by frequently washing out through the relief valve with excess oxygen. After that operation the subject was not allowed to breathe ordinary air until the whole of the oxygen series of tests was completed. During rests, and during the first part of a climb while the respiration was accelerating, he used the rescue apparatus. The routine was the same as that described above, the Douglas bag, however, being filled with a measured volume of oxygen, and the subject, on the word of command, changing rapidly from the rescue apparatus mouthpiece to that of the respiration apparatus, or *vice versa*.

The walking and running tests were made on a smooth, level concrete track at the Mine Rescue Station, Edinburgh.

The results of two such tests are set forth in Figs. 12 and 13. Fig. 14 is constructed from information relating to "C.G.D." and obtained from a paper by Haldane and Douglas(9); it is included for the sake of comparison. In condition (*a*), indicated on the graph by full lines, this subject was breathing normal air and walking on the laboratory floor, while in condition (*b*), indicated by chain dots, he was breathing air and walking in a level grass field. He did not carry a load; therefore, the consumption of oxygen and production of carbon dioxide are relatively less than those of the other subjects. Though the figures actually obtained in Haldane and Douglas' experiments were used in drawing the graphs, 25 p.c. has been added to "C.G.D.'s" oxygen consumptions in the following table to make them more comparable with those of the other men, who were carrying 43 lbs. each.

Tables III, IV and V, derived from the smoothed curves, state oxygen consumptions (expressed in litres per minute of dry gas at N.T.P.) of men walking and running on the flat and climbing the Burdiehouse incline:

Table III. Oxygen Consumption. Walking and running on the flat, carrying weight of 43 lbs.

	Miles per hour											
	Standing		1		2		3		4		5	
Subject	Br. air	Br. O_2	Br. air	Br. O_2	Br. air	Br. O_2	Br. air	Br. O_2	Br. air	Br. O_2	Br. air	Br. O_2
I	0·71*	0·68*	0·77	0·91	0·85	1·14	0·95	1·40	2·12	2·22	3·00	2·80
II	0·47	0·53	0·59	0·59	0·80	0·74	1·15	1·06	1·68	1·59	2·20	2·10
III	0·25	0·41	0·64	0·47	0·90	0·70	1·17	0·96	1·62	1·78	2·30	2·80
XIII	0·31	0·28	0·60	0·63	0·88	1·00	1·20	1·38	1·60	1·75	2·90	2·70
XVI	0·55	0·57	0·70	0·84	0·92	1·22	1·31	1·65	2·09	2·11	3·40	2·70
Average	0·40	0·45	0·65	0·69	0·87	0·96	1·16	1·29	1·82	1·89	2·80	2·60
C.G.D. (*a*)	0·40	—	0·60†	—	0·84	—	1·14	—	1·47	—	2·65	—
(*b*)	0·40	—	0·67†	—	0·98	—	1·33	—	1·99	—	3·17	—

* Unusually high: omitted in averaging.
† Interpolated from the graph.

Table IV. Oxygen Consumption. Climbing mine incline of 21°, carrying 43 lbs.

	Work done in foot-pounds per minute							
	Standing		3000		6000		9000	
Subject	Breathing air	Breathing oxygen	Breathing air	Breathing oxygen	Breathing air	Breathing oxygen	Breathing air	Breathing oxygen
I	0·62	0·57	1·08	1·37	2·02	2·17	3·10	3·00
II	0·42	0·40	1·14	1·19	2·20	2·55	—	3·40
III	0·35	0·41	1·14	1·09	2·19	2·05	—	—
XIII	0·47	0·45	1·40	1·24	2·56	2·37	3·10	2·60
XVI	0·45	0·47	1·24	1·24	2·02	2·02	2·80	2·85
Average	0·46	0·46	1·20	1·23	2·20	2·21	3·00	3·00

Table V. Oxygen Consumption. Climbing mine incline of 21°, carrying 43 lbs.

	Speed in miles per hour; slope measurement							
	Standing		0·5		1·0		1·5	
Subject	Breathing air	Breathing oxygen	Breathing air	Breathing oxygen	Breathing air	Breathing oxygen	Breathing air	Breathing oxygen
I	0·62	0·57	1·10	1·40	2·18	2·25	3·20	3·10
II	0·42	0·40	1·10	1·14	2·15	2·50	—	3·40
III	0·35	0·41	1·25	1·19	2·45	2·30	—	—
XIII	0·47	0·45	1·50	1·31	2·70	2·46	3·20	2·70
XVI	0·45	0·47	1·26	1·26	2·04	2·04	2·80	2·90
Average	0·46	0·46	1·24	1·26	2·30	2·31	3·10	3·00

Most economical rate of walking. Like a steamboat or an airship, a man has a most economical speed at which he goes furthest per litre of oxygen or per pound of fuel or food consumed. The data obtained yield the following information: "C.G.D.'s" most economical speed while breathing air and walking without burden on the laboratory floor, was four miles per hour, at which rate he moved 99 yards per litre of oxygen consumed. Walking without burden on grass, the same subject's most economical speed was three miles per hour, when a litre carried him 82 yards. With all the other subjects of Table III—loaded, as each of them was, with 43 lbs.—the most economical speed proved to be three miles per hour when breathing air, while, when breathing oxygen and similarly loaded, that rate was three miles per hour for I, II and III, and four miles per hour for XIII and XVI. It is apparent that increased difficulty of walking, whether due to the man carrying a weight or to lack of smoothness of the path, reduces the most economical speed.

The writer expresses his obligation and gratitude to Dr J. S. Haldane for the encouraging interest he took in these experiments and for his equally invigorating criticism. Mention must also be made of the loyal assistance given by Miss Elizabeth Gilchrist, M.A., B.Sc., and Mr David Penman, B.Sc., in conducting the experiments; of the painstaking work of the Physical Test Station Staff, and of the very willing help given by a great many mine officials, miners, soldiers, and others in the course of tests which were often of an arduous nature.

SUMMARY.

(1) Physical work is found by experience to be easier to unfit men when oxygenated air is breathed than when normal air is breathed, but no such difference is to be observed with fit men.

(2) When exertion of steadily increasing magnitude is undertaken, the expired-CO_2-percentage first rises and then falls. The load at which that percentage is a maximum is called the "crest load." It is shown that the crest load demarcates between normal loads and overloads. The demarcation line is not constant, and the circumstances causing movement of that line are discussed.

(3) If curves be drawn showing work done (abscissæ) and exhaled-CO_2-percentage (ordinates), (*a*) when the subject breathes air, and (*b*) when he breathes oxygen, the curves are found to coincide up to the crest where the man is very fit and to diverge widely when he is unfit, since the CO_2-percentage becomes much lower in the unfit when only ordinary air is breathed. A method of measuring fitness is described; it is based upon the experimental fact that fitness is inversely as the divergence of these curves.

(4) On an overload, even the fittest man derives benefit from breathing enriched air.

(5) The nature of the adaptation produced by physical training and by certain vocations is compared with that found to result from living at a high altitude. The bearing of the results upon the oxygen secretion question is considered and reasons are given for the acceptance of the secretion hypothesis.

(6) The benefit of breathing enriched air when doing physical work is limited to air containing about 60 p.c. oxygen. Enrichment above that proportion has no effect during exertion, even on very unfit persons.

(7) Tables are inserted setting forth the oxygen consumptions of numerous subjects while working the ergometer, while walking and running on the flat, and while climbing a mine incline of 21° slope, and the most economical rates of walking are shown for several subjects.

APPENDIX. *Description of subjects selected for illustration.*

Subject I (Fig. 3). Miner (repairer) working in steep seams; weight, 154 lbs., fitness 83 p.c.; the position of the "peak load" (7500–8000 ft. lbs. per min.) is higher than the average, indicating a man of good stamina.

Subject II (Fig. 4). Sedentary person; weight, 136 lbs.; fitness 46 p.c.; finds work much easier when breathing oxygen; oxygen-want apparent at relatively low rates of exertion.

Subject III (Fig. 5). Instructor at a mine rescue station; weight, 165 lbs.; fitness 80 p.c.

Subject IV (Fig. 6). Mine undermanager; weight, 168 lbs.; engaged in a mine working flat seams; fitness, 64 p.c.; oxygen-want becomes serious under 6000 ft. lbs. per min.

Subject VI (Fig. 7). Army recruit; previously a bank clerk; weight, 142 lbs.; fitness, 42 p.c.

Subject VIII (Fig. 8). Regular soldier; weight, 168 lbs.; instructor in physical drill; heavy weight lifter; athletic type; fitness, 100 p.c. Judging from his general behaviour while doing work (quite apart from the results obtained) he is the fittest man of the series. Breathing oxygen is not the least benefit until the crest load is exceeded; then it gives a gradually increasing assistance.

Subject IX (Fig. 9). Mine fireman or deputy, working in a flat-seam colliery; weight, 168 lbs.; fitness, 69 p.c.

Subject XIII (Fig. 10). First-class footballer; runner, jumper and all-round athlete; Army instructor in physical drill; weight, 158 lbs. Records were obtained of the subject in three conditions: (1) in good health; fitness, 70 p.c. (the ergometer results labelled (*A*) were obtained on that occasion); (2) in good health and after intensive training at Aldershot; fitness, 100 p.c.; and (3) in poor health; fitness, 55 p.c. The ergometer tests marked (*B*) and the climbing and walking tests were carried out with the man in the last condition. The low rate of breathing, small lung-ventilation, great depth of breathing and abnormally high CO_2-percentage level are remarkable features.

Subject XV (Fig. 11). Assistant instructor at a mine rescue station; weight, 147 lbs.; fitness, 63 p.c.

Subject XVI (Figs. 12, 13). Research assistant, sedentary habits; weight, 154 lbs.

C. G. D. (see p. 308), Fig. 14.

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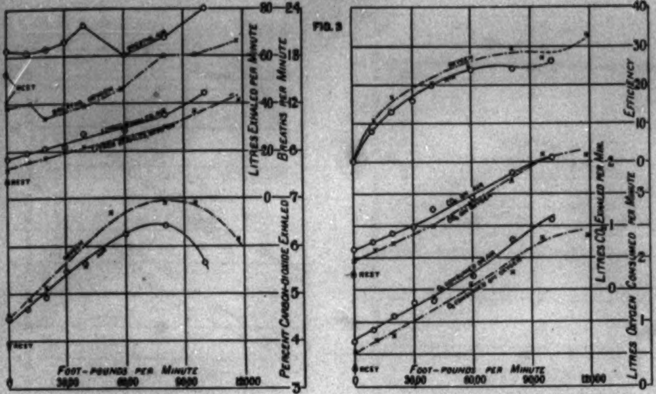
SUBJECT I



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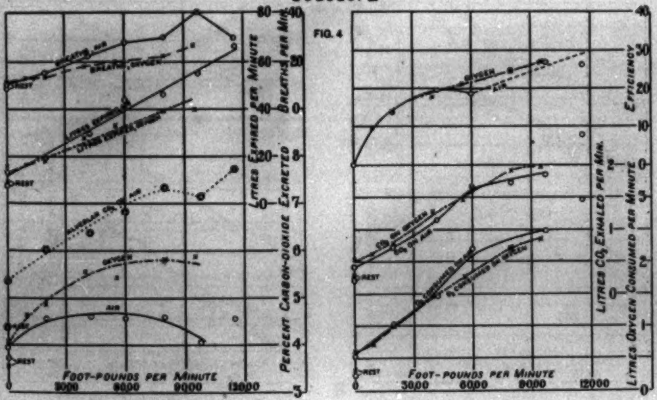
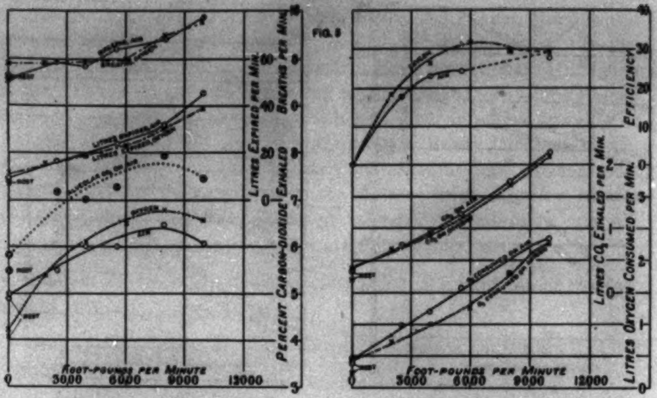


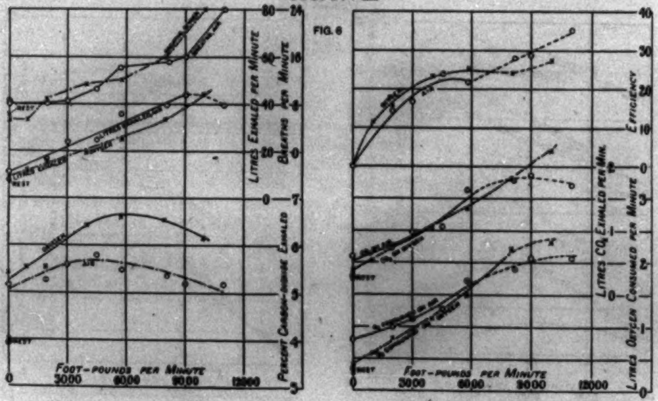
SUBJECT III

FIG. 5



SUBJECT IV

FIG. 6



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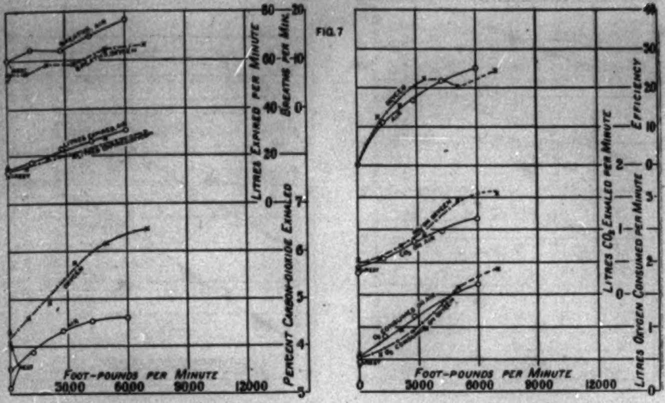


FIG. 7

SUBJECT VIII

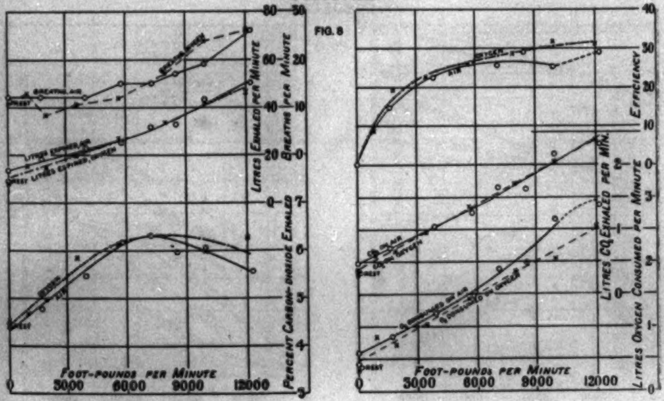
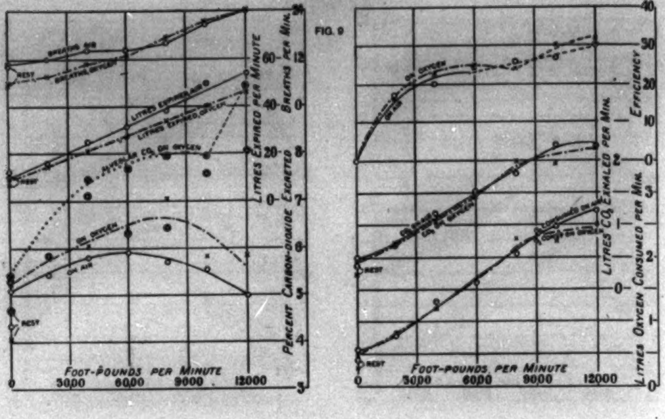


FIG. 8

SUBJECT IX



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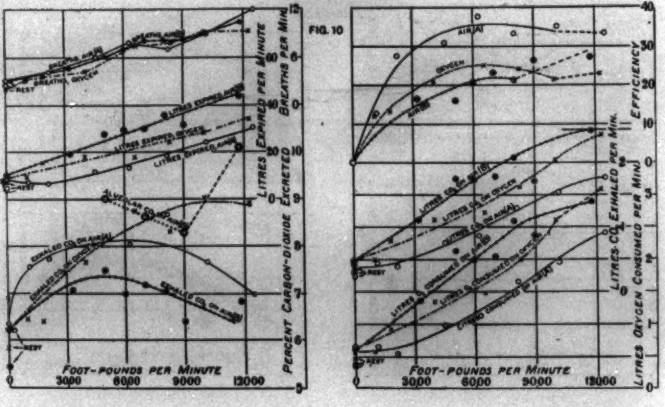
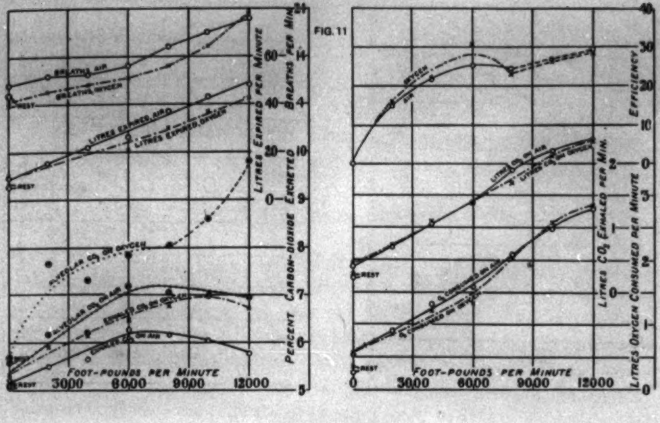


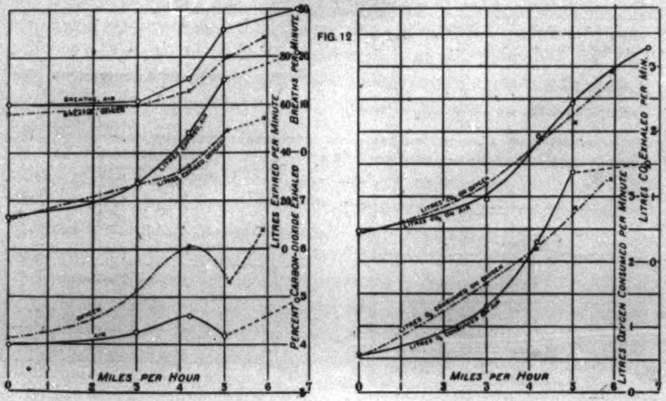
SUBJECT XV

FIG. 11



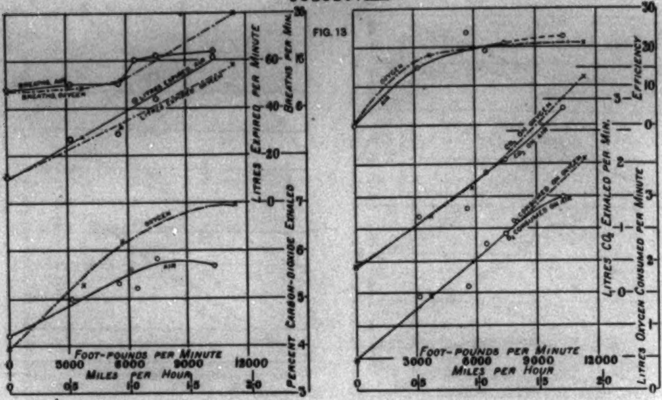
SUBJECT XVI

FIG. 12



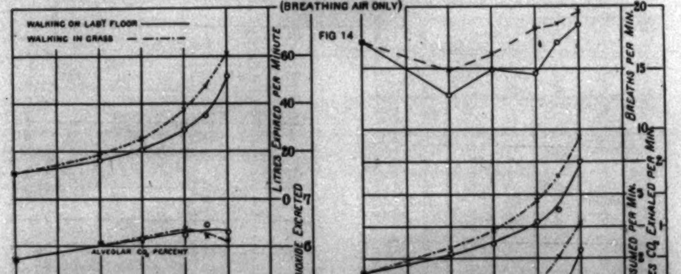
SUBJECT XVI

FIG. 13



SUBJECT-C.G.D.
(BREATHING AIR ONLY)

FIG 14



THE METABOLISM OF THE SALIVARY GLANDS.

I. The Relation of the Chorda Tympani to the nitrogen metabolism of the Submaxillary Gland. By G. V. ANREP.

(*From the Institute of Physiology, University College, London.*)

LUDWIG(1) and Heidenhain(2) found that in protracted secretion the submaxillary gland is gradually exhausted and that in consequence the percentage of organic solids secreted in the saliva diminishes as the secretion goes on. The histological observations of Heidenhain showed that in the mucous salivary glands of the dog, the mucin of the saliva arose from a clear substance in the cells which stained but feebly with carmine, and that the cells stained more deeply with carmine the greater the amount of the saliva secreted; he found further that during secretion the gland lost weight and that the percentage of solids in it decreased. Heidenhain concluded that the turning out of organic material by the cell was accompanied by the formation of fresh cell substance, but that this formation did not keep pace with the turning out. Actual comparison of the amount of the total organic substance lost and reformed was not made.

The point of view of Heidenhain was supported by Langley(3) chiefly on the ground that in glands in which an outer non-granular zone was formed during secretion—including the mucous glands—the amount of cell substance in the non-granular zone was greater than could be accounted for by the disappearance of the granules.

Pavlov(4) expressed doubt whether the increased affinity of the cell to carmine and the variations in the size of the cell as observed under the microscope are sufficient to make any definite conclusions about the output and the replacement of the organic material. Pavlov considered that only a direct observation of the nitrogen content of the gland is a suitable method for studying the metabolic processes during activity. He estimated the nitrogen content (*a*) of the gland on one side which had been left in the resting state; (*b*) of the gland of the opposite side which had been caused to secrete; (*c*) in the saliva secreted. He found that the active gland lost nitrogen, but that the loss was less than that of the nitrogen of the saliva. This was taken to mean that the gland took up protein during secretion. In a mean of ten experiments Pavlov found the amount thus stored up to be 26·7 p.c. of the nitrogen turned out of the gland (4 *a*, *b*). This taking up nitrogen was found to depend upon the nitrogen output, being considerably greater in experiments with a large secretion and a large nitrogen output (4 *c*). He found also that when the gland was left for eight hours after the stimulation the replacement of N was one-third, instead of about one-fourth. Since the secretion continued from about the first 50 minutes of this time, he concluded that the further increase was caused by nerve stimulation and that none occurred during rest. This fact led Pavlov to conclude that under normal conditions there must be some secondary stimuli which affect the gland after the end of the secretion and in some way stimulate the re-formation of the nitrogenous substances lost (4 *c*). In the experiments of Pavlov the secretion was excited in curarised dogs by stimulation of both sciatic nerves.

The results of Pavlov's experiments were confirmed by Verhovsky(5) who stimulated the peripheral end of the lingual nerve and the chorda and obtained in cases with large secretion a replacement of more than a half of the nitrogen output.

Langley(6) expresses some doubt whether the observations of Pavlov are correct "as the numerical results are not such as one would expect from the microscopical appearances of the gland cells."

Y. Henderson(7) performed similar experiments and found "that the secreting gland...remains in nitrogen equilibrium during secretion and tends to replace the loss during activity." In fact, the total of his nine experiments show a difference between the nitrogen of the nine active glands and the nine resting ones of only 1·4 p.c., although the saliva excreted contained 10 p.c. of the nitrogen of the gland. In three of these experiments the active glands were found richer in nitrogen than the resting ones. If these three experiments be excluded the figure of the replacement in the other six is about 32·4 p.c. of the loss which is close to the figure given by Pavlov. The experimental conditions varied in Henderson's experiments, and it seems possible that the deviation of the three experiments mentioned may have been due to this.

The problem of nitrogen metabolism is of importance for the proper understanding of the secretory process. The experiments described in this paper were performed with the object of studying these obscure points.

Comparative estimation of the nitrogen content and weight of the submaxillary glands on both sides.

The accuracy of the experiments greatly depends on the equality in nitrogen content of the glands on both sides of the animal. It has been found by Bidder and by Heidenhain that the left submaxillary gland is heavier than the right. Pavlov states that the glands may be heavier either on one side or on the other, but that their nitrogen content is remarkably concordant, the deviations being considerably less than 1 p.c. Henderson confirms this statement. As the question is of primary importance for the reliability of the experiments, 12 comparative estimations of the nitrogen in the glands were made by the Kjeldahl method. Most of the glands were taken from dogs used for other experiments but two special dogs were used. In all the dogs except one the digestive system had been at rest for some time before the glands were excised. The method of separating the glands in all the experiments described in this paper was the following: fine glass cannulas were tied in the *sublingual* ducts on both sides and two other cannulas were placed in the peripheral ends of both carotid arteries. The external jugular veins were then opened and normal NaCl solution was passed through the carotid arteries. When the outflowing fluid became colourless the perfusion was stopped. By means of a syringe a suspension of Indian ink was injected in the cannula of the sublingual ducts until the glands became distinctly stained. Then the submaxillary glands were dissected together with the sublinguals. The separation of the submaxillary from the sublingual is an easy matter if one uses this method of injecting Indian ink. Otherwise it is difficult to distinguish small lobules of the sublingual, which sometimes project into the body of the submaxillary. The separated gland was put into a watch glass and as far as possible liberated from the connective tissue. If one cuts with fine scissors along the duct, all the connective tissue of the hilus and that between the lobules with ducts and blood vessels together can be easily removed. When the gland is so prepared it completely loses its shape, it opens out and appears like a flattened disc. The glands when ready were weighed and their nitrogen was estimated.

There is no need to give the details of all these determinations as they showed complete concordancy. The total figures of the 12 determinations are:

The weight of the 12 right glands	41·3942 gr.
The weight of the 12 left glands	42·3614 ,,

A difference of 0·9672 gr.

The nitrogen content of the 12 right glands	...	1·1589 gr.
The nitrogen content of the 12 left glands	...	1·1608 „

A difference of 0·0019 gr. The maximal difference observed was about + 6 p.c. for the left gland in the dog which had been fed not long before the glands were removed. In this case the heavier left gland was richer in nitrogen by 0·0023 gr. which was a little less than 2 p.c. of the total nitrogen in the gland. The determinations justify the assumption that the submaxillary glands on both sides contained practically the same amount of nitrogen.

The effect of chorda stimulation on the total nitrogen metabolism of the submaxillary gland.

The first problem was to repeat the experiments of Pavlov, Verhovsky and Henderson in order to test their observations of an increased nitrogen intake during activity. Ten experiments were performed on dogs. Care was taken to have the digestive organs of the animals in resting condition. Water was given in abundance. Pure chloroform was used as an anæsthetic. As the object of these experiments was to investigate the uncomplicated chorda action, it was necessary to eliminate all stimuli which would affect the gland through the sympathetic nerve. The mere cutting of the vago-sympathetic in the neck could not be considered sufficient as there is always the probability of a stimulation of the sympathetic nerve endings by a discharge of adrenaline. For this reason in addition to cutting both vago-sympathetic nerves the suprarenal bodies were excised, either directly through the abdomen, or extraperitoneally. On the side of the gland which was taken for the control the chordo-lingual nerve was cut immediately after the dog was fastened on the table. The other chordo-lingual nerve was ligatured as deep as possible and prepared for stimulation. Fine glass cannulas were tied in both submaxillary ducts. When the whole preparation was completed a dose of morphia was injected subcutaneously and the anæsthetic changed for an E.C. mixture. Morphia and ether were not given before in order to avoid the stimulation of the salivary centre and not to increase the secretion of adrenaline any more than possible. If morphia is injected before the chorda tympani nerves are cut, there is always a large secretion from both glands, so that the control gland could not be considered as resting. The ligatured chorda-lingual nerve was stimulated with an interrupted current, a metronome being introduced into the circuit. The current used was very weak, hardly appreciable by the tongue, but was gradually increased towards the end of the experiment. The stimulation started from the end of the nerve close to the ligature, and the electrodes were gradually shifted along as the stimulated place lost its excitability. The chorda was not stimulated at any point nearer than ½ to 1 cm. from the gland in order to avoid a spread of current to the sympathetic fibres running with the artery to the hilus. The nerve was stimulated at intervals varying in different experiments from 3 to 15 minutes. Generally, the stimulation was stopped for a time when the secretion began to diminish during the stimulation. All necessary precautions were taken to keep the nerve moist and the animal warm. The time of stimulation varied from 1½ to 7½ hours. At the end of the experiment the amount of the secreted saliva was measured, the glands dissected as described above, weighed, and the nitrogen of both glands and of the saliva determined. The results of these ten experiments are presented in Table I, they were calculated in the following way. The nitrogen of the saliva represents the nitrogen output. The nitrogen intake of the active gland is given by subtracting the nitrogen of the resting gland from the total of the nitrogen in the saliva plus that in the active gland.

The percentage of the nitrogen output, the corresponding percentages of the nitrogen intake, and that of the actual loss by the secreting gland, calculated on the nitrogen of the resting gland are presented in Table II.

The secretion of the mucin nitrogen and non-mucin nitrogen.

The well-known fact, that in protracted secretion the saliva becomes less and less viscid and eventually appears as a watery fluid, shows that the excretion of mucin greatly diminishes. As the mucin is not the only nitrogenous compound excreted in the saliva, the question arises whether the excretion of the non-mucin nitrogen is parallel to that of the mucin excretion, or whether it is an independent one.

In order to determine the distribution of the mucin and non-mucin nitrogen in prolonged secretion, five experiments were performed. For the separation of the mucin from other organic and inorganic nitrogenous compounds the acetic acid method of precipitation of mucin was generally used. The saliva secreted was collected in separate portions from 5 to 25 c.c. each. The time of secretion of each portion was noted, and in each experiment the strength of the stimulation was altered in such a way as to maintain a constant speed of secretion throughout the experiment in order to avoid as far as possible the influence of the variation in speed upon the excretion of organic solids which was observed by Heidenhain. Each portion as soon as collected was diluted with

TABLE I.

Exp.	Weight of dog kgm.	Gland stimulated	Duration of the stimulation h.	m.	Volume of saliva c.c.	Weight of the gland in grams		Nitrogen in grams				
						Active	Resting	i Active gland	ii Saliva	iii Active gland + saliva	iv Resting gland	v = iii − iv The increase in N on the active side
1	9·0	Right	1	30	15	4·011	4·224	·0915	·0095	·1010	·0987	·0023
2	7·5	Left	2	30	20	2·588	2·924	·0649	·0101	·0750	·0712	·0038
3	9·0	Right	3	15	34	4·193	4·772	·0954	·0209	·1163	·1089	·0074
4	8·0	Left	4	30	43·5	2·915	2·976	·0591	·0173	·0764	·0706	·0058
5	8·25	Right	4	30	55	2·557	2·757	·0660	·0223	·0883	·0795	·0088
6	9·0	Left	5	00	65	2·946	3·245	·0798	·0279	·1077	·0958	·0119
7	8·5	Left	5	45	75	2·431	2·567	·0775	·0309	·1084	·0938	·0146
8	9·5	Right	6	00	90	3·621	3·977	·0799	·0345	·1144	·0990	·0154
9	12·5	Right	6	15	100	5·082	5·860	·1391	·0625	·2016	·1728	·0288
10	8·75	Left	7	30	150	3·107	3·557	·0722	·0432	·1154	·0907	·0247
Totals	90·00		46	45	647·5	33·451	36·859	·8254	·2791	1·1045	·9810	·1235

TABLE II.

Exp.	1	2	3	4	5	6	7	8	9	10	The mean
Percentage of N output	9·6	14·2	19·2	24·5	28·0	29·1	32·9	34·9	36·2	47·6	28·5
Percentage of N intake	2·3	5·3	6·8	8·2	11·1	12·4	15·5	15·5	16·6	27·2	12·6
Percentage of loss in N	7·3	8·9	12·4	16·3	16·9	16·7	17·4	19·4	19·6	20·4	15·9

distilled water to a volume of 100 c.c. An aliquot part of this diluted saliva was taken for the estimation of the total nitrogen (N/100 HCl used for titration) and another part was precipitated with acetic acid. The saliva before the precipitation was once more diluted with water, as it was found that the mucin precipitates far better if dilute.

After allowing the precipitate to shrink together into solid masses, which usually took ½ to 2 hours, the saliva was filtered and the nitrogen estimated in the filtrate. In two experiments the mucin was precipitated with very weak hydrochloric acid, as recommended by Hammarsten (8). The mucin was reprecipitated twice, finally filtered, and the non-mucin nitrogen estimated in the filtrate. The method of Hammarsten is not so good for the saliva mucin as it is for the mucin extracted from the gland, and a higher concentration of HCl has to be used to redissolve the saliva mucin than he advises for the gland mucin. Comparative determinations of the saliva treated with acetic acid and HCl gave sufficiently concordant results. Every collected portion of the secreted saliva was treated in the same way. Duplicate determinations were made in every case. Figures obtained in three of these experiments are given in Table III.

TABLE III. *The course of secretion of mucin nitrogen and non-mucin nitrogen in protracted secretion.*

Exp. 1.

c.c. of saliva	Mucin N in mgs	Non-mucin N
5	8·12	0·69
5	6·36	0·73
5	4·78	0·69
5	3·42	0·69
5	2·96	0·69
5	2·61	0·69
5	2·25	0·69
5	1·57	0·83
5	1·22	0·69
5	0·72	0·68
5	0·59	0·66
5	0·41	0·66
5	0·35	0·68
5	0·23	0·61
5	0·00	0·69
5	0·00	0·61
5	0·00	0·61
5	0·00	0·61
5	0·00	0·69
5	0·00	0·69
5	0·00	0·55
5	0·00	0·69
5	0·00	0·69
115	35·59	15·51

Exp. 2.

c.c. of saliva	Mucin N in mgs	Non-mucin N
15	21·24	2·86
15	11·89	2·70
15	5·31	2·54
15	0·96	2·86
15	0·64	2·70
15	0·48	2·62
15	0·00	2·86
15	0·00	2·62
120	40·52	21·76

Exp. 3.

20	25·04	3·72
20	12·74	3·93
20	6·45	4·01
20	1·77	3·93
20	0·50	3·93
100	46·50	19·52

The figures in Table III show that the excretion of mucin nitrogen follows a different course from that of the non-mucin nitrogen. As the secretion progresses the saliva becomes gradually poorer in mucin until no detectable traces remain. The diminution in the amount of mucin is comparatively rapid in the early stages of secretion, but afterwards it becomes slower and slower. For a considerable period after the secretion has been in progress only traces of mucin are secreted and finally they disappear completely. The non-mucin nitrogen follows quite a different course of excretion and proceeds at the same level from the beginning up to the end of the secretion. The figures of Exp. 1 are plotted as a diagram on Fig. 1 which makes the above facts more evident.

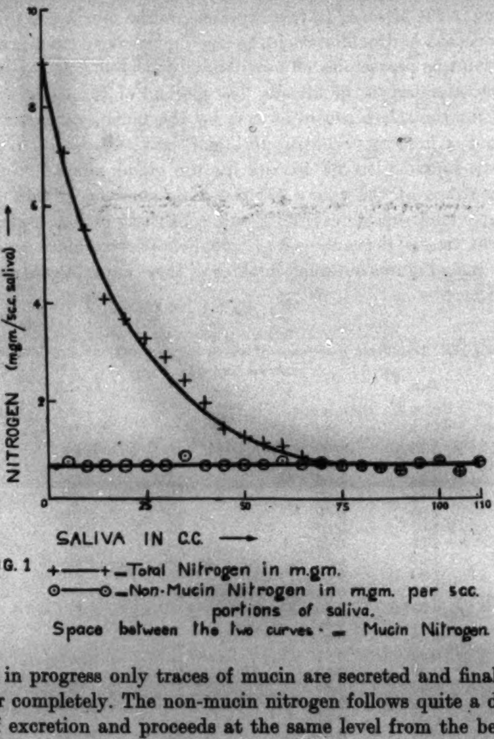


FIG. 1 +——+ – Total Nitrogen in m.gm.
⊙——⊙ – Non-Mucin Nitrogen in m.gm. per 5cc. portions of saliva.
Space between the two curves – Mucin Nitrogen.

On the grounds of these experiments it seems that the exhaustion of the gland affects only the mucin fraction, but not the non-mucin fraction of the nitrogen. That is, it would appear, that the mucin nitrogen is secreted from a store which can give only a limited supply and which is not replaced during secretion, on the other hand the non-mucin nitrogen is secreted from a store which is readily and quickly replaced from an apparently unlimited source during the process of secretion.

The relation of mucin nitrogen and non-mucin nitrogen to the nitrogen output and intake.

The figures given in Table II show that the intake of nitrogen is relatively greater the longer the secretion has continued. Seven more experiments were performed with the object of determining whether the nitrogen intake depends on the total nitrogen output or on the output of its two fractions.

In each experiment the whole saliva was collected together, measured and thoroughly mixed. One portion of this saliva was taken for the determination of the total nitrogen and another portion for the non-mucin nitrogen. Duplicate or triplicate determinations of nitrogen were performed in each case. The glands were dissected in the manner described above, weighed and their nitrogen determined as well. The results of these seven experiments are given in Table IV.

Attention should be given to the columns ii and vii and to the columns iii and viii. The column ii represents the figures of the non-mucin nitrogen secreted in the saliva, column vii the increase in nitrogen on the active side, that is nitrogen intake. The corresponding figure of these two columns are practically identical, at any rate, the differences are well within the limits of experimental error. On the other hand, the figures of columns iii and viii show that the loss in nitrogen by the active gland is equal to the mucin nitrogen excreted into the saliva.

On the ground of these experiments, the conclusion may be drawn that the increase in nitrogen on the active side is in some way correlated with the excretion of the non-mucin nitrogen and is quantitatively determined by the latter.

The non-mucin nitrogen of the saliva either represents a passive transport of some nitrogenous compounds from the lymph by the flow of water, or it may be due to an active secretion by the gland of some specific nitrogenous substances which are eliminated from the cells at a slow rate, and replaced at the same rate. Therefore the non-mucin nitrogen continues to appear in the saliva even when the mucin store has been used up. Further, every additional portion of saliva will add

TABLE IV. Weights in grams.

Exp.	Volume of saliva	i Active gland N	ii Non-mucin N in saliva	iii Mucin N in saliva	iv Total N in saliva = ii + iii	v Active gland + saliva N = i + iv	vi Resting gland N	vii The increase in N on the active side = v − vi	viii Actual loss in N by the active gland = vi − i	ix Difference between values in columns ii and vii and in iii and viii
1	25·5	·0643	·0040	·0078	·0118	·0761	·0723	·0038	·0080	± ·0002
2	40	·1397	·0125	·0322	·0447	·1844	·1706	·0138	·0309	∓ ·0013
3	55	·0672	·0074	·0146	·0220	·0892	·0811	·0081	·0139	∓ ·0007
4	60	·0766	·0123	·0200	·0323	·1089	·0969	·0120	·0203	± ·0003
5	65	·0770	·0126	·0190	·0316	·1086	·0952	·0134	·0182	∓ ·0008
6	100	·0554	·0122	·0133	·0255	·0809	·0694	·0115	·0140	± ·0007
7	115	·0746	·0167	·0179	·0346	·1092	·0934	·0158	·0188	± ·0009
Totals	460·5	·5548	·0777	·1248	·2025	·7573	·6789	·0784	·1241	∓ ·0007

some nitrogen to the active side and increase what has been called the nitrogen intake.

Table III shows that in chorda stimulation the percentage of mucin in the submaxillary saliva entirely depends on the stage of secretion. In the beginning of the secretion there may be about 0·16 p.c. of mucin nitrogen; but this figure quickly diminishes, so that after a protracted secretion, acetic acid does not give the faintest precipitate. On the other hand, if one considers the figures of the actual loss in nitrogen in experiments with complete exhaustion of the submaxillary gland, one finds that the percentage of the maximal loss is always very much the same, and varies between 19 and 21 p.c. This means that the submaxillary gland under the experimental conditions employed does not lose more than one-fifth of its nitrogen, and the whole of this nitrogen appears to be excreted in the form of mucin.

It may be concluded that the resting submaxillary gland of the dog contains about one-fifth of its nitrogen in a form which under chorda stimulation can be given off as mucin.

The non-mucin nitrogen has a more constant percentage in the saliva, the mean figure of 20 experiments being ·018 p.c. In 16 experiments the figures varied between ·013 and ·019 p.c., four experiments gave larger deviations, the smallest figure being about ·011 p.c. and the largest ·034 p.c. It appears that the larger glands secrete saliva with a larger percentage of non-mucin nitrogen.

As there is at least one factor in the nitrogen excretion in the saliva which has a nearly constant value, and practically depends on the mount of saliva secreted, it appears justifiable to plot the results of all these experiments so as to obtain three curves: (1) for the percentage of nitrogen output, (2) for the percentage of nitrogen intake = output of non-mucin nitrogen, and (3) for the percentage of actual loss in nitrogen by the gland = output of mucin nitrogen. The ordinates of this diagram represent the c.c. of saliva secreted. So that the whole diagram although constructed on a base of 18 experiments represents the behaviour of a single submaxillary gland. Too much importance must not be laid on this diagram as a representation of the true process, but as all the experiments were made under the same conditions and most of them on dogs of similar weight it seems such curves may help to represent it.

Fig. 2 shows that the curve representing the total nitrogen output at first rises steeply, but later straightens out. The excretion of mucin nitrogen, that is, the actual loss of nitrogen by the gland, after a step-like rise turns and runs horizontal. The excretion of non-mucin nitrogen, that is the increase in nitrogen in the active gland, has a course of a straight line which finally runs parallel with the curve of the total nitrogen excretion—as at the time of complete exhaustion it fully determines the total nitrogen output. The last figures plotted on Fig. 2

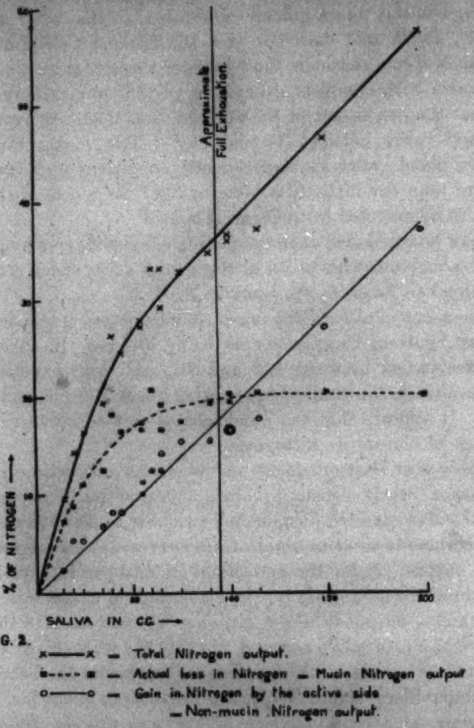


FIG. 2.

which correspond to a secretion of 200 c.c. were taken from another series of experiments not described in this paper.

The vertical line represents the place where the gland can be looked on as exhausted.

SUMMARY.

1. The older statement that during secretion produced by the stimulation of the chorda tympani the output of nitrogen by the submaxillary gland is greater than the loss by the gland is confirmed.

2. The excess of the nitrogen output over that lost by the gland increases with increase of output.

3. The output of mucin nitrogen, which as is known decreases during secretion, ceases altogether after a time, and is equal to the loss of nitrogen by the gland.

4. The output of the non-mucin nitrogen continues after the output of the mucin nitrogen has ceased, it proceeds on the same level throughout the secretion and is equal to the excess of output over the loss. Its mean percentage in the chorda saliva is ·018 p.c.

5. The results show that within the limits of experimental error the mucin of the saliva comes entirely from the mucin or mucinogen prestored in the gland, that the non-mucin nitrogen is derived from the body fluids and that no formation of mucin or increase of nitrogenous cell substance takes place during chorda stimulation.

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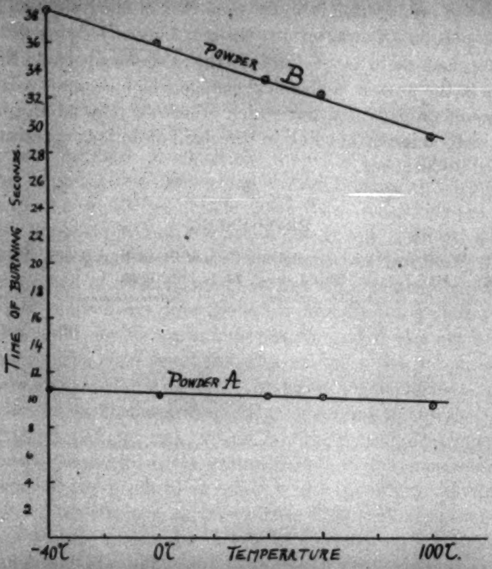
THE TEMPERATURE COEFFICIENT OF THE VELOCITY OF A NERVOUS IMPULSE. By A. V. HILL.

The phenomena of the passage of a nervous impulse have been compared to those of a chemical system, such as a train of gunpowder(1). Keith Lucas has shown that the velocity of a nervous impulse is increased 1·79 times by a rise of temperature of 10° C.(2), and it has been argued that this is a sign of the chemical nature of the changes underlying the propagated disturbance in nerve. In this argument a certain confusion tends to arise between the "velocity" of a chemical reaction, *i.e.* the rate of change of chemical substances, and the "velocity" of a wave, *i.e.* the rate of linear displacement of the point at which the chemical change is taking place. The slow burning of a train of compressed gunpowder is undoubtedly a chemical reaction, but the experiments described below show that a rise of temperature of 10° C. increases the velocity with which the wave of combustion travels by only a very small amount.

In 1917–18 Dr W. J. Goudie, then of University College, London, undertook, at the request of the writer, a series of experiments on the effects of a rise of temperature on the time of burning of a powder-ring fuse. A fuse consists of highly compressed gunpowder, forced into a groove in a metal ring, fired at one end by a detonator and at the other end itself firing a pellet by which the completion of its burning is determined. The time of burning is taken by one or more observers with stop watches reading to 0·02 second. The temperature of the powder is altered, between extreme limits of −40° C. and +100° C., downwards by pouring liquid air on the complete fuse, upwards by placing it in a water jacket. The temperature of the fuse is taken with a thermometer immediately before it is fired. The results of two sets of experiments on two widely different powders are shown in the figure. It is seen that in the case of one type of powder a rise of 140° C. decreased the time of burning of a given length of powder from 10·84 to 9·92 seconds, while with another type it decreased the time of burning of a rather greater length of powder from 38·5 to 29·3 seconds. The time of burning appears to be a linear function of the temperature so that, at 30° C., a rise of temperature of 10° C. increases the rate at which the wave of combustion is propagated 1·006 and 1·019 times respectively. If we were to trust to the temperature coefficient to decide whether the wave of combustion is a physical or a chemical one, we should unhesitatingly, and quite erroneously, conclude in favour of the physical theory. In actual fact the action at any given point is a chemical one, viz. a combustion, though the means by which it is transmitted is physical, viz. conduction of heat to contiguous particles of the powder. The chemical reaction may go on appreciably faster as the result of a rise of temperature, its "velocity" at one point may have the usual temperature coefficient of a chemical reaction: but if it be propagated by a physical occurrence its "velocity" of propagation may be practically unaffected by the rise of temperature.



The nervous impulse similarly consists of two separate things: the change at a given point, and the transmission of that change to a neighbouring point. It may be that the former is a reaction going on very rapidly, so that the velocity of propagation of the impulse depends mainly on the speed of transmission of some influence from point to point. Or it may be that the former change goes on relatively slowly, while the transmission of the influence exerted by the change completed at one point may be relatively rapid: in that case the effect of temperature on the velocity of propagation depends mainly on its effect on the local change. At present, however, we are ignorant of the relative importance of the two phases. The temperature coefficient found by **Keith Lucas** for the velocity of propagation is higher than that of most physical changes. We cannot conclude, however, that the reactions underlying the nervous impulse are chemical in nature but only that a chemical change interposes *somewhere* in the process, a chemical change which occupies a large part of the *time* of propagation, but which otherwise may be an important or a relatively unimportant link in the chain of actions constituting the propagated disturbance.

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STUDIES ON MUSCULAR CONTRACTION. II. The relation between the maximal work and the tension developed in a muscle twitch, and the effects of temperature and extension.

BY YASUKAZU DOI (JAPAN).

(*From the Physiological Laboratory, Cambridge.*)

IN a previous paper(1), in which the influence of temperature on the mechanical performance of muscle was studied, I estimated the mechanical performance of a contracted muscle indirectly by measuring the absolute tension developed on isometric contraction. It will be shown below that the former is a direct function of the latter, and until recently there was no better method of estimating the former than to measure the latter. A. V. Hill(2) pointed out that the work done by a muscle on isotonic contraction could not be a maximal one, so that it was not a measure of the mechanical activity of the muscle, while the potential energy thrown into an excited muscle on its isometric contraction was a reasonable measure of the mechanical activity. According to him this potential energy is about $\frac{1}{6}Tl$ gm. cms., where T is the tension developed on isometric contraction and l is the natural length of the muscle, so that T is a measure of the mechanical activity of a given muscle. But there remains some uncertainty as to whether T is still a measure of the mechanical activity when the muscle is extended to a comparatively large degree and also subjected to different temperatures, *i.e.* in the conditions of my experiments in Part I.

Recently A. V. Hill(3) established a method of measuring directly the maximal work done by a contracting muscle. By means of this method the mechanical performance of a muscle on contraction can be measured directly under variable conditions. At his suggestion, I have studied the relation between the maximal work done by a muscle on contraction and the tension developed in an isometric twitch at various extensions and temperatures; both contraction and isometric twitch resulting from a single induction shock.

Method of estimating the maximal work. The principle and construction of Hill's maximal work device have already been described by him (3). The details of use, however, have not yet been described, and therefore I give an example.

A double sartorius muscle of a frog (see Fig. 1) is immersed in oxygenated Ringer's solution at a given temperature, the lower end of the muscle is fixed to the bottom of the vessel, while the upper end is attached to the beam *B* of the instrument. The rider *w*, which is hung on the end of the other branch of the beam, will stretch the muscle, the extension of which is adjustable by means of a screw *S*, which supports the beam. When the muscle is stimulated by a maximal single opening shock, the weight *w* will be lifted for *h* cm., and thus the muscle will do work *wh* gm. cm. The moment of inertia MK^2 of the beam around its axis is adjustable by sliding the balanced masses *M* along it. If *a* be the distance of attachment of the muscle from the knife edge *A*, the equivalent masses ("E.M.") of the system is MK^2/a^2. The work done has a maximal value with a certain optimal value of *w*, and also with a certain optimal value of the "E.M." This maximal value of the work done may be called here the total maximal work. For example, the following figures were obtained with a muscle at an extension of 20 mm.

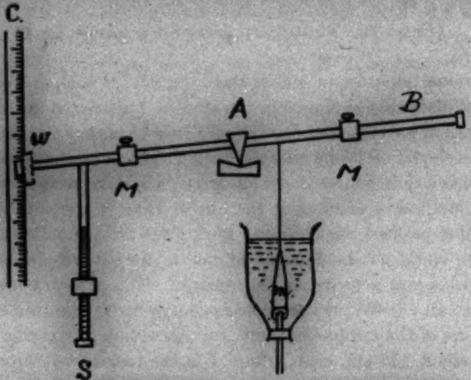

Fig. 1. A. V. Hill's maximal work device. Description see Text.

w (gms.)	E.M. (gms.) =	479	881	1314
·9	*wh* (gm. cms.) =	6·93	7·56	7·38
1·1		7·37	7·70	7·48
1·3		7·15	74·1	7·20

It is seen in the table that the total maximal work of 7·70 gm. cm. is obtained with $w = 1\cdot1$ gms. and E.M. = 881 gms. This total maximal work (W_t) is the sum of the following two kinds of work; (1) work (W) converted from new potential energy, liberated by the activity of the muscle on contraction, which we will call here "absolute maximal work," and (2) work (W_e) converted from a certain portion of elastic potential energy, which is possessed by the stretched muscle independently of its activity. To obtain (W), which is the real work done by the physiological activity of the muscle, (W_e) must be subtracted from the total maximal work (W_t). When the muscle shortens infinitely slowly (reversibly in the thermodynamical sense), (W_e) is equal to the total amount of the elastic potential energy possessed by the stretched muscle. But when the muscle shortens with a finite velocity, the conversion of the elastic potential energy into external work is irreversible, so that only a portion of the former is converted into the latter (W_e), the other portion of the former being degraded into heat by viscous process in the muscle (see Hill and Hartree(4)). The portion of the elastic potential energy, which will be converted into (W_e), decreases with increasing rapidity of shortening. Therefore (W_e) must be estimated by measuring the work done by the stretched muscle on releasing, when it is allowed to shorten (without excitation) at the same rate as that of the shortening in an actual twitch. This is obtained approximately as follows: it can be assumed that the rates of shortening on simple release and actual twitch are directly proportional respectively to the initial tension T_i, and to the total tension T_t developed on isometric contraction, and inversely proportional to the equivalent mass, against which the muscle has to pull. Now, in the example given above, recording the tension developed on isometric contraction at the same extension, it was found that the initial tension was 23·8 gms. and the total tension 77·0 gms. Thus, if the stretched muscle is released pulling against a system, the equivalent mass of which is

$$\text{optimal E.M.} \times \frac{T_i}{T_t} = 881 \times \frac{23\cdot8}{77\cdot0} = 272 \text{ gms.}$$

then its rate of shortening will be approximately the same as that with which the stretched muscle contracts on excitation, doing the maximal work of 7·70 gm. cms. as stated above. Actually releasing the stretched muscle (extension = 20 mm.) against this momentum (E.M. = 272 gms.) I obtained a work (W_e) of 1·06 gm. cms., lifting 0·2 gm. weight to a height of 53 mm. Then the absolute maximal work (W) which is done by the active process of the excited muscle in this example is

$$W = W_t - W_e = 7\cdot70 - 1\cdot06 = 6\cdot64 \text{ gm. cms.}$$

Other methods and procedure. The tension developed on isometric contraction is recorded by means of A. V. Hill's tension lever (cp. Hill(2)). The length of the stretched muscle is given in relative figures (which we may call the relative length), the length of the resting muscle being taken as unity. This is obviously necessary to make comparisons possible between long and short muscles.

Some experiments are made at a constant temperature (12° C.), estimating the absolute maximal work at different extensions of the muscle. The other experiments are made at two different temperatures (5° C. and 15° C.) and various extensions. The methods of keeping the muscle at a desired temperature is the same as that described in Part I. The muscle is kept at a certain extension and its absolute maximal work is determined at 5° C., then at 15° C. and again at 5° C. to make sure that no appreciable effect of fatigue or exhaustion spoils the result. This procedure is repeated at different extensions of the muscle.

Results and discussion. From three experiments, made at a constant temperature (12° C.), it is found that the absolute maximal work has its maximal value at a certain moderate extension. Before this optimal extension is reached, the absolute maximal work increases with the extension; beyond the optimal extension it decreases with increasing extension.

From five experiments, made at different temperatures (5° C. and 15° C. respectively), it is found that, at the same extension, the absolute work done at 5° C. is greater than that done at 15° C. The optimal extension appears to be a few millimetres longer at 15° C. than at 5° C. After this optimal extension, the decrease of the absolute maximal work with increasing extension is more rapid at 5° C. than at 15° C., so that at an extreme extension, the maximal work done at 15° C. may become larger than that at 5° C. Table I and Fig. 2 given here will be sufficient to represent the whole of the experiments made.

TABLE I.

Relative length	Absolute tension gms.		Maximal work gm. cms.	
	5° C.	15° C.	5° C.	15° C.
1·45	22·5	16·0	2·70	1·74
1·60	40·0	30·0	4·55	3·40
1·75	41·1	31·1	4·92	3·72
1·90	31·9	31·9	3·82	3·62
2·05	18·2	21·8	2·72	2·82

In the procedure of finding the work (W_a), the initial and total tensions developed on isometric contraction are estimated at every extension. From these data we can find the relation between the absolute tension developed on isometric contraction and the extension of the muscle at different temperatures. The results obtained are quite similar to those given in my previous paper, so that it is unnecessary to give them here.

Now, in investigating the relation between the maximal work done in a twitch by the activity of a single muscle (*W*) and the tension developed on its isometric contraction *T* under a variety of conditions, it is unnecessary to do more than to determine the variations of *W*/*T*. If, however, we desire to compare the value of *W*/*T* obtained for one muscle with that obtained for another, it is clearly necessary to introduce some factor depending on the length of the muscle, for, with a muscle 2 cms. long, (*W*) will be twice as large as with a muscle 1 cm. long, while *T* will be unaffected. The simplest way to do this in the case of a muscle of uniform cross section such as a sartorius muscle is to calculate the work done per unit length of muscle, and to compare this with the tension developed in it; in other words, to use, not *W*/*T*, but *W*/*Tl* as a basis of comparison, *l* being the unextended length of the muscle. It is unnecessary to introduce any factor depending on the cross section

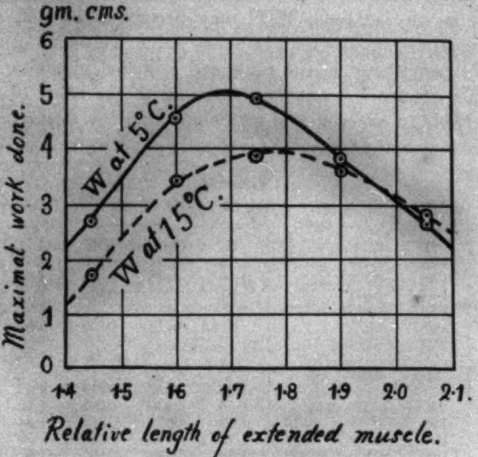

Fig. 2.

of the muscle, as this affects both (W) and T alike, leaving their ratio unaffected. The employment of W/Tl has the further advantage that (W) and Tl are both of the dimensions of work and therefore W/Tl is a pure number not depending upon the units of force and length employed.

The ratios W/Tl obtained in all experiments done are summarised in Table II, from which it is seen that W/Tl is, relatively speaking, a fairly constant quantity in each muscle, independently of its extension, thus confirming the use by A. V. Hill of the tension developed as a measure of the mechanical performance of the muscle. Only when the extension is very extreme W/Tl may become abnormal, sometimes larger, sometimes smaller than its mean value. Those abnormal figures are put in parentheses in the table and excluded in taking the mean value. This irregularity may be due either to the effect of fatigue or more probably to experimental errors affecting the estimation of (W). In a single muscle the ratio W/Tl is slightly larger at 5° C. than at 15° C.

TABLE II.

Relative length	W/Tl	Relative length	W/Tl		Relative length	W/Tl		Relative length	W/Tl	
I	12° C.	III	12° C.		V	5° C.	15° C.	VII	5° C.	15° C.
1·42	·0437	1·28	·0350		1·35	·0472	·0400	1·45	·0600	·0545
1·57	·0445	1·43	·0385		1·50	·0476	·0405	1·60	·0570	·0565
1·68	·0454	1·58	·0400		1·64	·0462	·0396	1·75	·0600	·0600
1·80	·0450	1·73	·0405					1·90	·0600	·0570
1·87	(·0312)	1·88	·0375		Mean	·0470	·0400	2·05	(·0700)	(·0650)
		2·03	(·0295)							
Mean	·0447							Mean	·0593	·0570
		Mean	·0383		VI	5° C.	15° C.			
II	12° C.				1·28	·0422	·0379	VIII	5° C.	15° C.
1·62	·0385	IV	5° C.	15° C.	1·50	·0456	·0409	1·33	·0454	·0377
1·77	·0400	1·39	·0505	·0318	1·63	·0478	·0421	1·44	·0523	·0420
1·92	·0380	1·52	·0509	·0336	1·76	·0465	·0414	1·56	·0535	·0424
2·07	·0450	1·66	·0500	·0350	1·89	·0495	·0446	1·67	·0546	·0454
Mean	·0404	Mean	·0505	·0335	Mean	·0463	·0414	Mean	·0515	·0419

It was thought at first that it might be better to employ, not the unextended length l, but the extended length l' in the formula W/Tl, but it was found that for a given muscle W/Tl was much more nearly constant than W/Tl': in the table, therefore, I have given only the former.

SUMMARY.

At a constant temperature the maximal work done by a muscle excited by a single shock has a maximum value with a moderate initial extension of the muscle. Before this optimum extension is reached the absolute maximal work increases, and beyond this optimum it decreases, with increasing extension.

The absolute maximal work done in a twitch at the same extension of a single muscle is greater at a lower temperature than at a higher.

The optimum extension is shorter at a lower temperature than at a higher.

The ratio W/Tl, where W is the maximal work done in a contraction, T is the tension developed in a similar isometric twitch and l is the natural length of the unexcited muscle, is a constant independent of the extension of the muscle: it is slightly smaller at a high temperature than at a low one.

In conclusion I wish to express my warmest thanks to Dr A. V. Hill for his kindness in allowing me to use his maximal work device, and also to him and to Mr W. Hartree for their valuable advice in performing these experiments. I also take this opportunity of expressing my thanks to Prof. Langley, in whose laboratory these experiments were made.

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(4) A. V. Hill and W. Hartree. Phil. Trans. Roy. Soc. **210** B. p. 153. 1920.

VARIATIONS IN ALVEOLAR CARBON DIOXIDE PRESSURE IN RELATION TO MEALS[1]. By E. C. DODDS.

(From the Bland-Sutton Institute of Pathology, Middlesex Hospital.)

THE investigation described below arose in the following manner. More than a year ago a gas analysis apparatus for the estimation of oxygen and carbon dioxide was being tested; atmospheric air was, of course, used as a source of oxygen, and it was thought that the alveolar air of one person would provide an approximately constant concentration of carbon dioxide. However, ten analyses on samples taken at different times of the day showed a range of about 8 mm. in carbon dioxide pressure. As such differences could not be attributed to error in the analyses, it seemed that the pressure of alveolar carbon dioxide was less constant than one had been accustomed to think. On considering the factors which could theoretically produce such variations the taking of food seemed to be the most probable; on putting this to the test, a rise in CO_2 pressure after a meal, and a fall later, were found to occur. The matter was not pursued further until quite recently, when the above observations were mentioned to Dr Haldane; he had noticed such variations and thought the matter would repay thorough investigation. The experiments described below were therefore carried out.

Methods. All the samples were collected by the Haldane-Priestley method, and all analyses were carried out on the Haldane apparatus. Each point on the following curves is the mean of four analyses, namely, of two inspiratory and two expiratory samples; one pair of samples was analysed in a large and one in a small Haldane apparatus; these had been thoroughly calibrated and compared. Observations were made on twelve normal persons and on one who had undergone the operation of gastrectomy.

The first subjects were three healthy men, of ages between 20 and 40. (1) In A. N. K. (Fig. 1 and Table I) the alveolar CO_2 was determined by the above method half-an-hour before his lunch, taken between 11.15 and 11.45 a.m., and at half-hourly intervals afterwards until the CO_2 pressure had regained its original level, which occurred at 2.30 p.m. The figure shows that the CO_2 pressure rose nearly 5 mm. during the hour following the meal; it then fell to nearly the same extent below the original level, and had reached this level again about three hours after the meal.

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[1] A report to the Medical Research Council.

This series of changes might, of course, be a diurnal variation independent of the taking of food. Accordingly, observations were made over the same portion of the day, but lunch was deferred until 3.15 p.m. It will be seen that the pressures remained within a range of only 1 mm. during the whole four hours from 11 to 3, while the meal at 3.15 was followed by a rise and fall of the same type as that observed after the customary lunch hour. (2) A confirmatory result was obtained in an experiment of exactly the same type on another subject E. L. K. (Table I), though the full series of changes following the later meal was not followed. (3) Four series of observations were made on E. C. D. In three of these the usual lunch was taken, and the alveolar CO_2

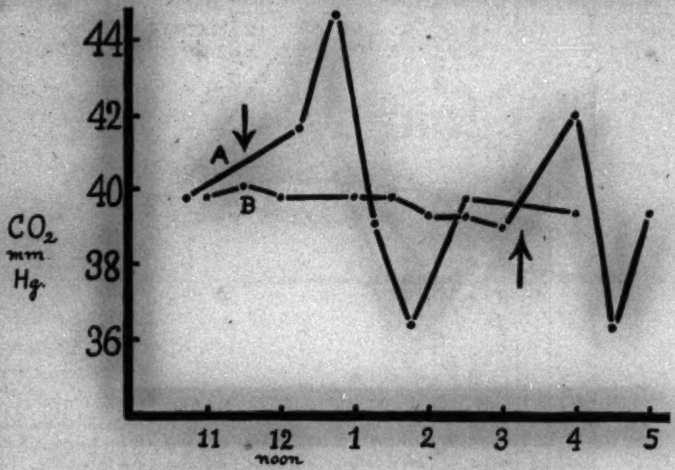

Fig. 1. Observations on A. N. K. Series A, meal from 11.15 to 11.45. Series B, meal from 3.15 to 3.30. Arrows indicate time of meal.

TABLE I. *Alveolar* CO$_2$ *pressures of three normal subjects, and of gastrectomy case.* M indicates time of meal.

E. C. D.

With meal						Without meal	
Series A		Series B		Series B$_2$		Series C	
Time	CO$_2$ mm.	Time	CO$_2$ mm.	Time	CO$_2$ mm.	Time	CO$_2$ mm.
12	39·8	12.30	39·3	12	44·2	12.30	36·1
12 to 12.30	M	12.45 to 1	M	12 to 12.30	M	1.30	36·3
1	45·3	1.30	41·1	1	46·2	2.30	36·1
2	36·7	2.10	38·0	1.30	42·8	3	36·9
2.30	34·5	2.45	35·0	2	42·3	4.30	36·1
3.30	40·4	3.45	39·6	3	38·2		
4	41·2						

E. L. K.

Usual meal Series A		Deferred meal Series B	
Time	CO$_2$ mm.	Time	CO$_2$ mm.
12	41·3	11.30	36·6
12.15 to 12.45	M	12	36·7
1	42·7	1	36·1
1.30	46·6	1.30	36·1
2	42·0	2.30	36·1
2.30	38·1	3	36·5
3	40·1	3.30	36·5
3.30	40·4	3.40 to 4.10	M
		4.30	40·8
		4.45	42·1

G. A. (gastrectomy case).
Usual meal

Series D		Series E	
11.30	39·7	11.40	38·3
12 to 12.30	M	12 to 12.15	M
1	40·1	12.45	39·1
2.30	39·8	1.15	39·0
3	38·2	1.45	36·4
4	34·1	2.15	34·5
		2.45	34·0
		3.15	38·7
		3.45	38·3

A. N. K.

10.45	39·8	11	39·8
11.15 to 11.45	M	11.30	40·1
12.15	41·7	12	39·8
12.45	44·7	1	39·8
1.15	39·0	1.30	39·8
1.45	36·4	2	39·4
2.30	39·8	2.30	39·4
4	39·4	3	39·0
		3.15 to 3.30	M
		4	42·1
		4.30	36·3
		5	39·4

pressure rose 5·5, 1·8, and 2·0 mm. respectively on the three occasions; on the fourth no food was taken throughout the period of observation, and the CO_2 pressure remained within a range of 0·8 mm. (Fig. 2 and Table I). It seemed clear then that these changes were associated with the ingestion of food.

Observations were then made on a further series of healthy persons, of ages ranging from 15 to 50, before and after their usual mid-day meal. All the data obtained are given in Table II, and as many as can be drawn without confusion are shown in Fig. 3 together with one of the series (B_2) from E. C. D. mentioned above. It will be seen that in

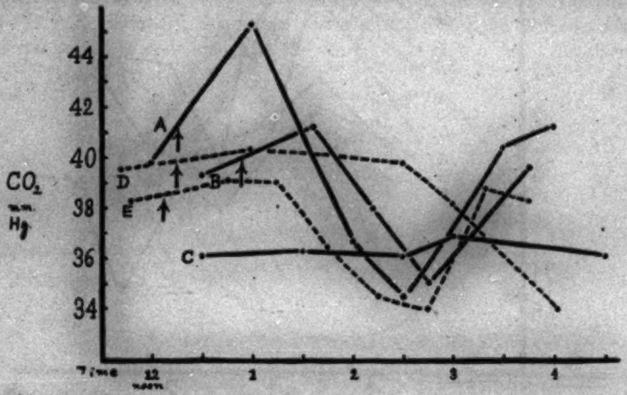

Fig. 2. Continuous lines. Observations on E. C. D. (normal). Series A and B with meal. Series C without meal.

Broken lines. Observations on G. A. (gastrectomy case). Series D and E with meal.

TABLE II. *Alveolar carbon dioxide pressures of healthy persons before and after the mid-day meal.*

	Before meal	After meal										
Hours	½	½	¾	1	1¼	1½	2	2¼	2½	2¾	3	3½
H.	42·8	45·9	—	39·4	—	37·5	—	39·0	—	42·1	—	—
J. D.	42·1	45·1	45·8	—	43·2	38·2	42·1	—	42·4	—	—	—
G.	38·4	40·5	—	38·6	—	36·3	37·8	—	38·6	—	—	—
M.	37·6	41·6	—	39·7	—	—	—	—	33·0	—	37·6	37·6
J.	46·3	49·8	—	—	—	—	—	—	—	—	—	—
T.	39·1	44·8	—	—	—	—	—	—	—	—	—	—
Mr.	39·7	45·7	—	—	—	—	—	—	—	—	—	—
A. N.	37·2	40·2	—	—	—	—	—	—	—	—	—	—
McK.	39·2	43·8	—	41·9	—	39·6	36·7	—	39·2	39·9	—	—

every case the meal is followed by a rise in the CO_2 pressure, and wherever the observations are continued over a sufficiently long period a subsequent fall and return are seen. The highest point appears always to be reached within 30 to 45 minutes after the end of the meal; the greatest increase observed was 6 mm. (in Mr.) and the lowest 1·8 mm. (on one occasion in E. C. D.). The lowest point is reached 1½ to 2 hours after the end of the meal except in one case (M.), in which the fall lasts 2½ hours. The range of fall observed is about the same as that of the rise, namely,

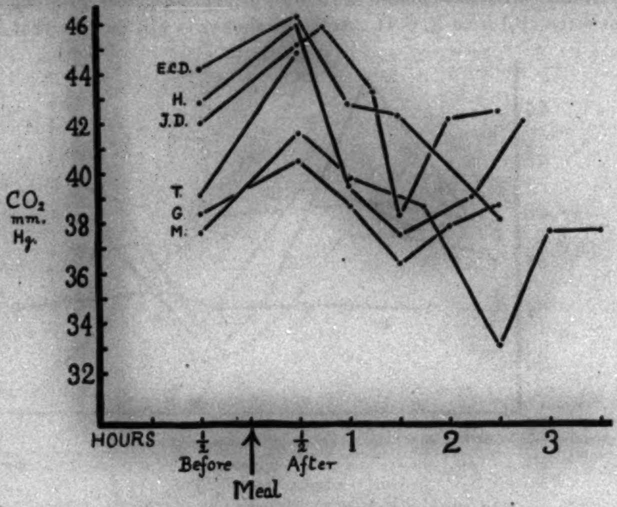

Fig. 3. Alveolar CO_2 of six normal persons before and after the mid-day meal.

from 2 to 6 mm. The diphasic type of change was found in all the cases that were observed for a sufficiently long time.

Since the rise in CO_2 tension occurs during the time when the secretion of gastric juice is taking place, the question arose whether the two changes were associated. Through the kindness of Mr Gordon Taylor, F.R.C.S., it was possible to make two series of observations on a man (G. A.) from whom the greater part of the stomach had been removed eight months before, in Feb. 1920, on account of severe gastric ulceration; he had made an excellent recovery, and was leading an active life as a salesman. The part removed from the patient consists of at least seven-eighths of the stomach together with the pylorus and the duodeno-jejunal flexure which had been anastomosed with the stomach at a previous operation (Fig. 4). Thus the only portion of stomach wall which the patient still possessed was that in the immediate neighbourhood of the cardiac orifice; this was joined to the jejunum, with which the duodenum was also brought into connection. The pancreatic juice and bile could thus enter the intestine.

The results given in Table I and Fig. 2 show that the CO_2 pressure increased after the meal by 0·4 mm. on the first occasion and by 0·8 mm. on the second, whereas no normal person showed a rise of less than 1·8 mm. (see E. C. D. Series B); this suggests very strongly that the rise seen in a normal person is associated with the secretion of gastric juice. Possibly even the small rise of 0·4 to 0·8 mm. would not have occurred if the stomach had been removed completely at the operation. It is noteworthy that this subject showed the later changes in CO_2 pressure, namely, the fall followed by the return to the original level, in just the same way as a normal person; this affords strong evidence that these changes are associated with digestive processes in portions of the alimentary canal below the stomach. In Fig. 2 are included for comparison three series of observations on a normal person (E. C. D., Table I) in two of which (A and B) the ordinary lunch was taken, while in the third (C) no food was taken throughout; in this last series the CO_2 pressure remained almost constant (between 36·1 and 36·9 mm.).

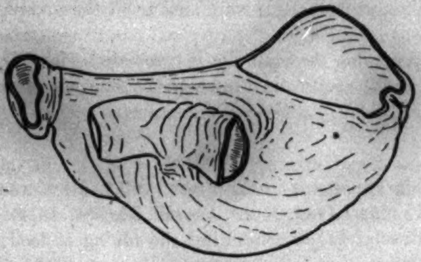
Fig. 4.

The application of this method to pathological cases is already under trial in this laboratory, and some interesting results have been obtained. The later changes in alveolar CO_2 pressure after a meal may perhaps throw light on processes such as those of pancreatic secretion which are less accessible to investigation than those taking place in the stomach.

Finally, I have to express the debt that I owe to Dr E. L. Kennaway for valuable advice during the work, and for help in the consideration of the results.

SUMMARY.

In a series of normal persons the alveolar carbon dioxide pressure was found to show the following changes after a meal: (1) a rise of from 2 to 6 mm. within the first half or three-quarters of an hour; (2) a subsequent fall of about the same amount (2–6 mm.) below the original level; (3) a return to this level.

In a man from whom the greater part of the stomach had been removed the rise after a meal was very small in amount (0·4 to 0·8 mm.) while the subsequent changes were similar to those seen in a normal person.

It seems probable that the rise is associated with the secretion of gastric juice, and the subsequent fall with the later processes of digestion.

Addendum.

Whilst this Paper was in the Press my attention was drawn to a paper by Higgins (*Amer. Journ. Physiol.* **34**. 114. 1914). He noticed the rise in alveolar CO_2-tension after the taking of food, but did not describe the subsequent fall and return. He regards the rise as associated with drowsiness and vaso-dilation, but his discussion of the matter is hard to follow.

NOTE ON THE SUPPOSED IDENTITY OF THE WATER-SOLUBLE VITAMIN B AND SECRETIN.

By G. V. ANREP and J. C. DRUMMOND.

(From the Institute of Physiology, University College, London.)

In a recent paper Voegtlin and Myers(1) put forward a theory that the antineuritic vitamin and secretin are possibly one and the same substance. This conclusion is based on the following observations: (i) Active vitamin preparations from brewer's yeast when injected intravenously cause an increased pancreatic secretion and bile flow; (ii) Secretin solutions may show considerable antineuritic properties; (iii) Inactive vitamin preparations from yeast retain their depressor effect on blood pressure but do not increase pancreatic secretion and bile flow; (iv) An extract of the duodenum of a polyneuritic cat had no effect on pancreatic secretion; (v) The two substances show similar chemical and physical properties.

An examination of the protocols of the experiments carried out by Voegtlin and Myers makes it apparent that their results are insufficiently conclusive to warrant the suggestion that secretin and vitamin B are identical.

In the first place, an increase in bile flow is not a good test, since it is not specific to the action of secretin, and largely depends on the capillary pressure and blood flow through the liver. The three injections of the active substance and one of the inactive did not yield concordant results. The active yeast preparation gave on one occasion an increase, once a doubtful effect, and once no effect at all, whilst the inactive preparation nearly doubled the bile flow in an animal in which the bile flow was not constant. The experiments on the action of the secretin on the bile flow do not add anything to the facts which are already familiar.

In the case of the pancreatic secretion it is well known that extracts of different organs may cause a small pancreatic secretion when injected intravenously, but this effect cannot be mistaken for that which follows the injection of an extract of intestinal mucosa. It is also quite probable that yeast extracts may have a feeble effect on the pancreatic secretion similar to that possessed by the extracts of organs other than the intestinal mucous membrane.

The injections of the active yeast preparation in Voegtlin and Myers' experiments gave in one case 27 drops, and in another 12 drops, in 30 minutes as compared with 476 drops in 40 minutes after the injection of 1 c.c. of secretin. In another case the difference was not so striking. It is not stated by these authors whether they ligatured the pylorus in their experiments. If not, their results may perhaps be explained by the fact that injections of the yeast extract are usually followed by a definite increase in peristalsis and in the case of large doses by cramps. Therefore, if the reaction of the stomach was even faintly acid, inaccurate results would be obtained unless the pylorus had been ligatured.

When we turn to the experiments in which the secretin preparations were found to have a curative action on polyneuritic pigeons little support is found for the idea that vitamin B and secretin are identical. Most normal organs contain small amounts of the antineuritic factor so that it is not surprising that highly concentrated extracts of mucous membrane were found to relieve the symptoms in pigeons.

In order to test the statement, however, a few experiments were carried out.

A highly active yeast extract (B 1) was prepared by extraction of a concentrated preparation of autolysed yeast with alcohol. After removal of the alcohol by evaporation in a vacuum the residue was dissolved in distilled water. This preparation was found to possess a high potency when tested on rats and pigeons. Yeast extracts B 2 and 3 were preparations of the vitamin B which had been subjected to slightly more purification. They were still powerfully active when tested by biological methods.

Exp. 1. Dog 8·5 kg. Chloroform-ether anæsthesia. No secretion of pancreatic juice was observed during an initial period of 10 minutes. The following injections were then made, a period of 10–15 minutes being allowed to pass between the cessation of secretion and the injection of another fraction.

Injection	Time of first drop	Total secretion	Time
3 c.c. yeast extract B 1	2 min. 4 sec.	2 drops	13 min.
2 c.c. secretin	46 sec.	54 „	21 „
3 c.c. yeast extract B 1	15 min.	1 „	15 „
5 c.c. yeast extract B 1	4 min. 20 sec.	4 „	19 „
2 c.c. secretin	37 sec.	61 „	25 „

Exp. 2. Dog 9·25 kg. Chloroform anæsthesia, secretin prepared from another normal dog. During the initial period of 10 min. there was no secretion of pancreatic juice.

Injection	Time of first drop	Total secretion	Time
3 c.c. yeast extract B 3	5 min. 2 sec.	27 drops	17 min.
3 c.c. secretin	37 sec.	99 „	28 „

Pylorus tied, weak solution sodium bicarbonate injected into duodenum followed by an interval of 45 minutes.

3 c.c. yeast extract B 2	10 min.	4 drops	55 min.
3 c.c. yeast extract B 3	15 min.	1 ,,	15 ,,
3 c.c. secretin	49 sec.	118 ,,	37 ,,
3 c.c. yeast extract B 3	2 min. 43 sec.	3 ,,	16 ,,
3 c.c. yeast extract B 2	13 min.	1 ,,	13 ,,
10 c.c. yeast extract B 3	8 min.	7 ,,	41 ,,

Severe cramps followed this injection, the heart nearly stopped, blood pressure fell almost to zero and recovery required about 50 minutes.

3 c.c. secretin	53 sec.	128 drops	38 min.
3 c.c. yeast extract B 3	—	—	—
3 c.c. yeast extract B 2	3 min. 15 sec.	1 ,,	14 ,
3 c.c. secretin	47 sec.	115 ,,	34 ,,

These experiments show that there is a very great difference between the effects of injecting secretin extracts and extracts known to be rich in the vitamin B.

Attention was then turned to the statements of Voegtlin and Myers regarding the absence of secretin from the intestines of cats which have been deprived of the vitamin B.

By following the method described by Voegtlin and Lake(2) in which cats are fed on meat which has been subjected to the action of dilute alkali at high temperatures, it is easy to produce a condition resembling the so-called polyneuritis of pigeons. A cat which had developed this condition after three weeks' feeding on the vitamin-free meat diet was used for the next experiment.

Exp. 3. Cat, adult. Rectal temperature 34·6° C. Ether anæsthesia. No secretion during initial 20 minutes. 10 c.c. of 0·3 p.c. HCl solution injected into the duodenum caused a pancreatic secretion which lasted about 15 minutes. A further 10 c.c. of the acid solution injected into the duodenum produced the first drop of pancreatic juice in 5 min. 20 sec. and a total secretion of 36 drops in 14 minutes. 1 c.c. of a secretin solution from a normal cat (secretin 1) produced the first drop in 34 sec., and a total secretion of 21 drops in 10 minutes. The intestines were then removed and a secretin preparation made from the mucous membrane (secretin 2).

The following results were obtained in the tests of these two preparations:

Injection	Time of first drop	Total secretion	Time
1 c.c. secretin 2	37 sec.	33 drops	10 min.
1 c.c. secretin 2	30 ,,	30 ,,	10 ,,
1 c.c. normal secretin 1	28 ,,	33 ,,	10 ,,
1 c.c. secretin 2	29 ,,	46 ,,	10 ,,
1 c.c. normal secretin 1	31 ,,	45 ,,	10 ,,

In every case a period of 10 minutes elapsed between the cessation of secretion and the next injection.

This experiment shows that in the condition produced in the cat by prolonged deficiency of the vitamin B secretin can still be extracted from the mucous membrane of the intestine, and that pancreatic secretion may be aroused by the injection of extracts of this tissue, whether derived from the polyneuritic animal or from a normal one.

A second cat which was placed upon the deficient diet at the same time developed the typical symptoms on the 25th day. On the following day the symptoms were much graver, the body temperature had fallen to 32·8° C. and the cat was unable to stand. A dose of secretin representing 5 gm. of mucous membrane of a normal rabbit was administered by the mouth. The condition of the animal did not improve but became steadily worse, until it finally died 15 hours after the administration.

The experiments in this work were carried out with crude secretin and vitamin B extracts, whereas in some of the experiments of Voegtlin and Myers preparations were used which had been subjected to a certain amount of purification by chemical methods. In fact, the authors regard the similar chemical properties of the two substances as evidence in favour of the theory that they are identical. It is very doubtful, however, whether this supposed similarity of properties is of any significance. One of us has had considerable experience in attempts to isolate the vitamin B by chemical methods and has come to the conclusion that very little reliance can be placed on the various methods of purification which have as yet been described. It is certain that our present knowledge of the chemical and physical properties of both secretin and vitamin B is insufficient to justify the conclusion that they are the same. In fact, we know that they are different in one important respect. Vitamin B has a powerfully curative action on polyneuritic animals when given by the mouth, whereas secretin has no action on the secretion of pancreatic juice when administered in this manner.

CONCLUSIONS.

1. Extracts prepared from yeast which show marked growth-promoting and antineuritic properties have no specific action on pancreatic secretion, and are not similar in this respect to secretin.

2. The pancreas of a cat showing typical symptoms induced by a diet deficient in the vitamin B responds in a normal manner to secretin.

3. Secretin can be extracted from the mucous membrane of the intestines of cats showing the so-called polyneuritic condition to a very marked degree.

4. The suggestion advanced by Voegtlin and Myers that vitamin B, or the antineuritic vitamin, is identical with secretin is not supported by experimental evidence.

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(1) **Voegtlin and Myers.** J. Pharm. and Exp. Ther. 13. p. 301. 1919.
(2) **Voegtlin and Lake.** Amer. J. Physiol. 47. p. 559. 1919.

ON A PROBABLE ERROR IN DETERMINATIONS BY MEANS OF THE HYDROGEN ELECTRODE. By C. LOVATT EVANS.

(*From the National Institute for Medical Research, London.*)

It has been recently pointed out by the writer (1) that the results of determinations of the hydrogen-ion concentration of the blood by means of the colorimetric method recommended by Dale and Evans (2), are not in agreement[1] with those of other observers who employed the electrometric method: the values obtained by the former method are approximately p.H 0·2 higher than those given by the hydrogen electrode, or by calculations based on constants derived from electrode determinations. As the reason for this discrepancy was obscure, it was necessary directly to compare the two methods, in order to find, if possible, under what conditions it appeared. The present communication deals with these comparisons, and with various other considerations bearing on the question. Although perhaps some doubt still remains as to the finality of the conclusions which will be presented here, reasons will be given for doubting the validity and explaining the difficulty of determinations of the reaction of carbonate-holding solutions by the gas-chain method.

Methods. Colorimetric determinations of the reaction of blood were made exactly as described by Dale and Evans; when bicarbonate solutions were under investigation the solution, at the requisite CO_2 tension, was generally transferred direct to the comparator vessel and at once covered with a layer of liquid paraffin, dialysis being omitted. When no electrometric determination was required the fluids were brought into equilibrium with the required CO_2-air mixture in a Barcroft saturator, and the actual tension of CO_2 at the end of equilibrium was determined by analysis of a sample of the gas by the small Haldane apparatus. For the electrometric measurements the type of glass-stoppered hydrogen electrode described by **Michaelis**(3) was used, the positive pole being provided by the *n*/10 or by the saturated KCl-calomel electrode (made according to **Michaelis**). In preparing fluids at definite CO_2 tensions the electrode was connected with the saturator as shown in Fig. 1. The whole apparatus, with the contained fluid, was twice or three times evacuated with a Fleuss pump and filled with hydrogen washed with alkaline pyrogallate, potassium permanganate and mercuric chloride solutions. After the final filling with hydrogen the requisite amount of carbon dioxide prepared from marble and HCl and washed with $NaHCO_3$ solution was added, and atmospheric pressure established by addition of more hydrogen.

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[1] The agreement between the results of our method and those by the electrometric method which was claimed in the paper by Dale and the present writer was principally due to the fact that our determinations were made at room temperature, whereas the calculated curve of Parsons was one for bicarbonate solution at 38°, as was also the case for the direct observations of Milroy on ·02 m. $NaHCO_3$ and ·18 m. NaCl. In our determinations with the latter solution moreover, it is quite possible that the bicarbonate was a little too weak because the solution was prepared by direct weighing and was not checked by analysis as the plain bicarbonate solution was.

The whole apparatus was rotated horizontally. After equilibrium had been reached and atmospheric pressure readjusted (if necessary) by addition of more hydrogen, it was easy so to manipulate the apparatus as first to fill the electrode vessel with the fluid, and then to pass up into it a bubble of the gas of a convenient size. After withdrawal of a sample of the gas for analysis, and of portions of the fluid for the colorimetric determination, the (meanwhile stoppered) electrode was connected up exactly as described by **Michaelis**. Definitive potentials were obtained almost at once provided the gas was free from oxygen; when this was not the case, there was at first a somewhat lower potential than that finally obtained after one or two hundred inversions.

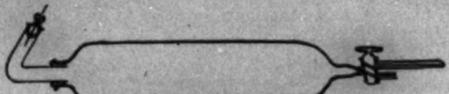

Fig. 1. The Saturator-electrode.

Alkali reserves were determined according to **Van Slyke**(4) and oxygen capacities by the Barcroft-Haldane method. Corpuscular volumes were obtained by measurements from blood centrifuged in glass capillaries.

Comparison of the two methods. The extent of the difference between the results of the two methods is illustrated by Fig. 2, which represents CO_2-reaction curves for goat's blood at 20° C., and also by Fig. 3 which gives the curves for my own blood at 38° C. determined by the indicator method, for comparison with the curves made by **Parsons**(5) and **Hasselbalch**(6) on their own bloods at the same temperature. It should be pointed out that the results of the indicator method, when neutral red (turning point p.H 7·4) is employed, are liable to a much greater error for values of p.H greater than eight or less than seven than for reactions lying between these limits, and little stress can be laid on any values which are beyond this, the most sensitive range. For the determination of the reaction of blood of p.H greater than 7·8, phenol red which is half dissociated at p.H 7·9 (Clark and Lubs(7)) could be used with advantage, though, as will appear presently (p. 359), the results with pure bicarbonate solutions would be incorrect. When the alkaline end of the indicator curve for blood is disregarded, it is seen that this graph is in each instance situated about p.H 0·2 higher than the corresponding electrometric curve, so that there is no doubt that the discrepancy is a real and regular one.

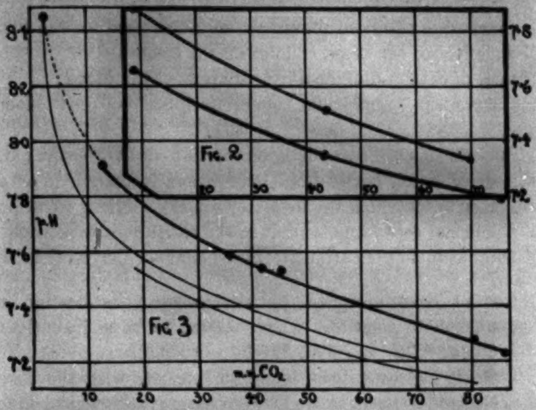

Fig. 2. Carbon dioxide-reaction curve for Goat's blood at 20° C. Alkali reserve of whole blood = 59 p.c. Corpuscular volume = 41·1 p.c. Alkali reserve of plasma at 45 mm. CO_2 = 69 p.c. Oxygen capacity of blood = 15·8 p.c. Upper curve is for colorimetric method with neutral red: lower curve electrometric.

Fig. 3. Carbon dioxide-reaction curves of human blood. Upper curve is for blood of C.L.E. at 38° C., done colorimetrically. Alkali reserve of blood = 60·3 p.c.: of plasma at 45 mm. CO_2 = 63·7 p.c. Corpuscular volume = 33·1 p.c. Oxygen capacity = 16 p.c. Middle curve is that of Parson's blood, and lowest one that of Hasselbalch's blood, by the electrometric method.

Now the dialysate from blood, which contains bicarbonate, ·85 p.c. chlorides and other salts, together with carbon dioxide, but no protein, is just such a solution as should give reliable results with well-known indicators like neutral or phenol red, and certainly should not when investigated by the hydrogen electrode give values for H-ion concentration some 60 p.c. greater than those found by the use of indicators; yet this is what happens in the present instance. In other words, of a blood dialysate and a given phosphate solution which both give the same tint with an indicator, the former has, according to the hydrogen electrode which is usually accepted as the final court of appeal, a H-ion concentration some 60 p.c. greater than the latter.

Discussion of the cause of the discrepancy. The following among the possible causes of this anomalous behaviour will be discussed seriatim:

(1) Inaccurate standard phosphate solutions used for the titration.

(2) An effect of the blood colloids on the electrode.

(3) Loss of carbon dioxide during dialysis of the blood.

(4) Incomplete equilibrium of electrolytes between blood and dialysate.

(5) Specific effect of carbonate, bicarbonate or CO_2 on the indicator: or a salt error much larger than that generally assumed: or a large personal error in matching the indicator solutions during titration.

(6) Abnormal contact potentials in the electrometric method.

(7) Some influence of carbonate, bicarbonate or CO_2 on the electrode.

(1) was at once excluded by electrometric verification of the standard phosphate solutions, which proved to be sufficiently correct ("6·5" = 6·516; "7·5" = 7·500; "10·5" = 10·555).

(2) Colloids have not been found to interfere with the use of the hydrogen electrode, except by slightly increasing the technical difficulties. In any case (2) and also (3) and (4) can be conveniently investigated by employing a solution of sodium bicarbonate instead of blood, and omitting the dialysis. This might also give some indication as regards (5).

For these experiments I used plain aqueous ·02 m. sodium bicarbonate solution at 20° C. under varying tensions of CO_2, and for the colorimetric method employed two indicators, neutral red and phenol red. The electrometric curve which was obtained agreed roughly with that given by McClendon(8) and based on the linear relation between *p*.H and log . CO_2 tension. The results, given numerically below are graphically represented in Fig. 4.

Disregarding for the moment the results obtained by the use of phenol red, it is seen that the discrepancy is still present and of the same magnitude as in blood.

The suggested possibilities numbered (2) and (4) at once fall out as of no moment. Loss of carbon dioxide is much reduced by the omission

TABLE I. Comparison of electrometric and colorimetric observations of the reaction of ·02 m. $NaHCO_3$ solution at 20° C.

	p.H				
	Titration				
CO_2 mm.	Neutral red	Phenol red	Electrometric	Discrepancy between N.R. and El.	
8·93	8·23	8·04	7·96	·27	mean = ·20
21·8	7·70	7·42	7·55	·15	
33·3	7·57	7·28	7·34	·23	
46·0	7·37	7·17	7·16	·21	
68·9	7·19	6·97	7·07	·12	

of the dialysis, and since the same results are given even when dialysis is carried out exactly as for blood, it would seem that (3) is also of no importance in practice: the same conclusion is also indicated by the fact that the electrometric and neutral-red curves do not diverge appreciably at high CO_2 tensions.

(5) is a suggestion which is not so readily eliminated, and the difficulty is not diminished by comparison of the results obtained by the two indicators, for those obtained by the use of phenol red lie slightly on the acid side of the electrometric curve, in spite of the probability that this indicator is liable to no other limitations than would appear to apply to the use of neutral red. It certainly does not seem likely that carbonic acid would exert any effect on either indicator apart from that which would be exhibited by any other acid of the same dissociation constant under the same conditions of concentration and temperature. Nor does the fact that neutral red is a basic indicator, while phenol red is a salt of a relatively strong acid seem to be capable of explaining the difference in their behaviour.

When the dialysate from blood was used for the colorimetric method, the same results were given by both phenol red and neutral red. In order to ascertain whether it is the presence of the chloride which makes this difference to the phenol red readings, I used a solution of bicarbonate and chloride, a few electrometric measurements being also made. The solution chosen was ·02 m. $NaHCO_3$ and ·18 m. NaCl in aqueous solution: these strengths were chosen because a similar solution was investigated by Milroy(9) (at 38° C.: mine was at 20° C.). For some of the colorimetric observations rosolic acid was used in addition. Table II gives the results, which are presented graphically in Fig. 5. It may be mentioned that the results are not greatly different if, instead of sodium chloride, potassium chloride of the same molar concentration is used, such differences as were observed being little beyond the limits of experimental error.

Hasselbalch(6) has shown that the p.H of a bicarbonate solution as determined by the H-electrode rises about 0·12 when the temperature is changed from 18° to 38° C. (see also **McClendon**(8), p. 272) so that by

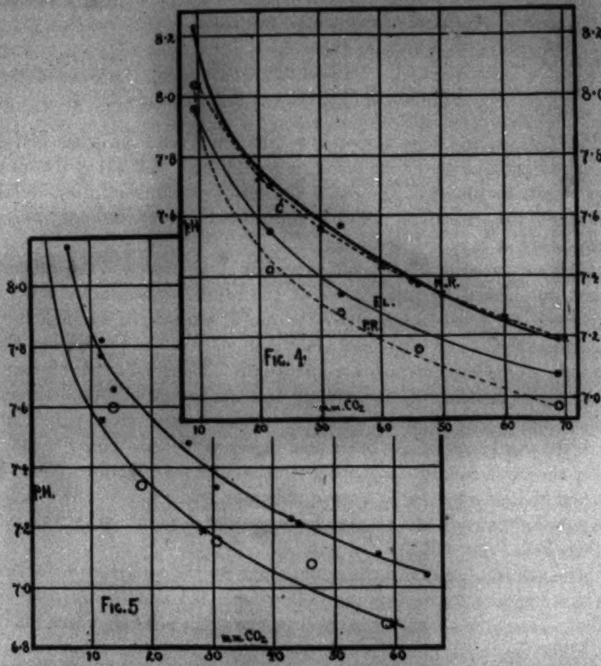

Fig. 4. Carbon dioxide-reaction curves for ·02 m. sodium bicarbonate at 20° C. *N.R.* = Neutral red curve. *C.* = Calculated curve. *El.* = Electrometric curve. *P.R.* = Phenol red curve.

Fig. 5. Carbon dioxide-reaction curves for ·02 m. sodium bicarbonate plus ·18 m. sodium chloride (or KCl). Upper curve colorimetric. Lower curve electrometric. The crosses on the latter represent determinations by Milroy (1917) modified in accordance with the different temperature (see text).

subtracting 0·1 from **Milroy's** results, his curve can be compared with mine; and some of his points are accordingly shown on the curve with this alteration.

The results given by three indicators are now identical, and the

TABLE II. Reaction of sodium bicarbonate (·02 m.) and sodium chloride (·18 m.) solution at varying tensions of carbon dioxide.

		Colorimetric		
mm. CO_2	*p*.H N.R.	P.R.	R.A.	*p*.H electrometric
6·5	8·13	—	—	—
11·5	7·77	—	—	—
13·9	7·66	7·67	(?) 7·64	7·60
18·8	—	—	—	7·34
26·0	7·48	—	—	—
30·5	7·33	7·34	7·31	7·15
42·8	7·23	—	—	—
44·0	7·21	—	—	—
46·1	—	—	—	7·08
57·2	7·11	—	—	—
58·5	—	—	—	6·88
65·4	7·04	—	—	—

indicator curve lies, as before, higher than the electrometric one. May we conclude from this that phenol red now gives correct values, or must it be supposed that all three indicators are wrong because they do not give results in agreement with the electrode?

The more nearly two solutions agree in chemical composition, the more likely it will be that when they give the same colour with an indicator, their H-ion concentrations are equal. As a possible method of deciding the point therefore, solutions were made up which differed but little from the original phosphate standards with which the titrations were made, and upon which standards the different indicators agree with each other and with the hydrogen electrode. Some of the *m*/15 phosphate of *p*.H 7·5 was therefore added to small amounts of sodium carbonate: in another experiment CO_2 was passed into the solution.

TABLE III. Comparison of reactions of phosphate-carbonate solutions.

	p.H by titration				
Solution used	N.R.	P.R.	R.A.	Electrometric	Discrepancy
Phosphate + *m*/300 Na_2CO_3	7·78	7·79	—	7·67	·12
Phosphate + *m*/150 Na_2CO_3	8·23	8·23	—	8·05	·18
Phosphate in equilibrium with 3 p.c. CO_2 ...	7·09	7·09	7·09	—	—

The results given by the different indicators are in agreement, and are again higher than the electrometric determinations. If we take these indicator readings as correct, it follows that the low values for *p*.H given by phenol red with dilute solutions of pure bicarbonate are not reliable, but are vitiated either by some circumstance which diminishes the dissociation constant of the indicator, or bicarbonate, or by one which increases the dissociation of carbonic acid—it would be mere speculation to guess which.

(6) Abnormal contact potentials, due to abnormal mobility of the HCO_3'-ion do not seem likely to account for the difference, since according to **Walker** and **Cormack**(10) the ionic conductivity of this ion has the very moderate value of 38.

(7) We now come to the last of the suggested possibilities, namely that there is an error inherent in the hydrogen electrode in presence of bicarbonate solutions.

Relation between the properties of platinum black and the electrode error in bicarbonate solutions. Putting aside the remote possibility that carbon dioxide sets up its own potential in the presence of the platinum, there are two other possibilities worthy of consideration. The first of these is that carbon dioxide, being a readily liquefiable gas, is adsorbed on to the platinum black, and thereby reduces the concentration of hydrogen upon it. The hydrogen adsorbed would then behave as an amalgam behaves on dilution; when the electrolytic solution pressure is proportional to the concentration of metal in the amalgam, dilution gives the same result as a rise in the concentration of the ions in the solution. This possibility is supposed to have been tested by **Höber**(11) and by **Farkas**(12). **Höber** used the chain

$$H_2 + CO_2/0{\cdot}1n\ H_2SO_4 + 1{\cdot}2\ NaCl/1{\cdot}2\ NaCl\ HgCl/Hg$$

and found that the effects of CO_2 were merely those of an indifferent diluent gas. I have also tried elements similar to those of **Höber**, but without finding that CO_2 made any appreciable constant difference: I used ·1*n* and ·001*n* HCl in presence of KCl, but found the diffusion potentials very troublesome: the greatest difference in E.M.F. was 5 mv. (once only), and it is quite possible that this was due to diffusion potential. But in any case the question cannot be solved by the use of half-cells of this type, because in such acid solutions the adsorption of CO_2 might be much reduced, and what we require is a cell that has a H-ion concentration of the same order as that of blood. There does not seem to be any direct way of experimentally testing this point, and none but an experimental investigation can help us. The fact that the discrepancy appears to be no greater at high than at low carbon dioxide tensions is certainly against the supposition that CO_2 is specifically adsorbed.

The second possibility seems, from the chemical point of view, to be the more likely. In the presence of such an efficient catalyst as platinum black the reduction of carbonate to formate, according to the reaction

$$CO_2 + H_2 = H\,.\,COOH \text{ or } NaHCO_3 + H_2 = HCOONa + H_2O$$

seems not beyond the bounds of possibility or even of probability.

I have endeavoured to test this possibility experimentally in the following way:

To 25 c.c. of 2 p.c. aqueous sodium bicarbonate solution 0·5 gram of recently well-ignited 20 p.c. platinised asbestos (Merck) was added: the mixture was placed in an atmosphere of hydrogen, which was frequently renewed, for 36 hours at room temperature, and finally heated to body temperature for one hour. The entire mixture was then treated with dilute sulphuric acid until distinctly acid to litmus, and at once distilled. The first 3 c.c. of distillate was faintly acid to litmus. Portions of it were made faintly alkaline with ammonia, and then boiled to expel the latter. Silver nitrate solution was then added: there was no colour change in the cold, but on heating for a few moments a brown turbidity appeared: this was readily soluble in dilute nitric acid in the cold, and was presumably free silver.

In one experiment the colour of the reduced silver solution was comparable with that given by 1: 40,000 formic acid solution, and in another where the action had only proceeded for 16 hours in the cold, with 1: 70,000. Blanks gave no result, nor was any result obtained in an experiment in which the hydrogen was led into a bicarbonate solution which was gradually heated to boiling during one hour.

Although the amount of reducing substance formed in the reaction was very small, the results of this experiment are of considerable theoretical significance, since if the conclusion indicated is the correct one, it may be taken as showing that the hydrogen electrode is incapable of giving correct results in the presence of carbonate or bicarbonate solutions.

After the above experiments had been carried out, I found by reference to some papers which were indicated to me by Dr Harold King, that the reaction is already well known. Deville and Debray(13) in 1874 showed that finely divided rhodium decomposes formic acid into hydrogen and carbon dioxide: Zelinsky(14) found the same to apply to palladium, while Blackadder(15) showed that this catalysis was retarded by alkali and also demonstrated that active rhodium has a more positive potential against formic acid than the inactive metal. Proof that the reaction was reversible was furnished by Wieland(16) in 1912 by the preparation of traces of formic acid from palladium, hydrogen and carbon dioxide; the most convincing evidence, however, was that of Bredig and Carter(17) who showed that under certain conditions as much as 75 p.c. of the calculated yield of formic acid was obtainable from bicarbonate solutions by the action of hydrogen under pressure in presence of palladium. The only possibly new chemical fact which arises out of my own experiment then, is the not surprising one that a similar catalysis takes place in the presence of platinum, under conditions which reproduce fairly closely those which obtain in the hydrogen electrode when blood or bicarbonate solution is under examination.

In exactly what manner the catalytic production of formic acid vitiates the results of electrode determinations, is uncertain; on the one hand, it might be held that the local establishment of a balanced reaction would result in the development of a definite local acidification, and thus account for the constancy of the discrepancy; on the other hand it would not be unreasonable to attribute the phenomenon to the existence of a reduction potential.

Calculation of the hydrogen ion concentrations of bicarbonate solutions. In his pioneer treatment of the question of the equilibrium between carbonic acid and bicarbonate L. J. Henderson (18) took as the dissociation constant of carbon dioxide in aqueous solution the value determined by Walker and Cormack (*l.c.*) by the conductivity method, while the ionisation of sodium bicarbonate was estimated to be about 0·8 in accordance with that of other salts of strong bases with weak acids. In the calculation of the reaction of blood or bicarbonate solutions so much in vogue at the present time, however, it is customary to employ constants derived from electrometric observations, such as those given in the careful work of Hasselbalch (*l.c.*), or the very thorough theoretical treatment by Parsons (19) based on his own observations and on those of other workers including Hasselbalch. Hasselbalch's constant $p.K_1$, which is used in his formula

$$p.H = p.K_1 + \log \frac{3.8s}{p.a},$$

where s = the alkali reserve,
p = the pressure of CO_2,
a = solubility of CO_2,

is equal to $p.K + \log.\delta$, where $p.K$ = the exponent of the dissociation constant of CO_2, *i.e.* the apparent dissociation constant of aqueous carbonic acid, and where δ = the degree of dissociation of sodium bicarbonate.

It cannot be too strongly emphasized that in such a calculation as this, we are only concerned with the *apparent* dissociation constant of carbonic acid, *i.e.* with the equation

$$CO_2 + H_2O \rightleftarrows (H_2CO_3) \rightleftarrows H^{\cdot} + HCO_3{}'.$$

Now if we take, as Hasselbalch does, values for δ obtained from conductivity measurements (by Walker and Cormack), and then proceed to determine, as he did, the value of $p.K_1$ from electrometric measurements this value for ·02 m. $NaHCO_3$ (where δ = ·851) works out at 6·38, whence $p.K$ = 6·45, or in other words, the apparent k for

$$H_2CO_3\ (38°) = 3{\cdot}55 \times 10^{-7},$$

whereas Walker and Cormack (*l.c.*) found $3{\cdot}04 \times 10^{-7}$ at 18° C., which according to Henderson (*l.c.*) would become about $4{\cdot}2 \times 10^{-7}$ at 38° C.

This agreed also with the value determined by **Henderson** and **Black**(20) by a chemical method. But **Parsons**(19) has already criticised the values of $p.K$ obtained by **Hasselbalch**, and pointed out that his formula is only applicable if the concentration of CO_2 is expressed, not in moles, but in half moles, which is indeed what the formula given above actually gives. If molar concentrations of CO_2 were used in the Hasselbalch formula, then his value for k as determined by the hydrogen electrode would be about $7 \cdot 1 \times 10^{-7}$ (*i.e.* $p.K = 6 \cdot 15$), which is very much higher than the value obtained by the other method ($4 \cdot 2 \times 10^{-7}$).

The reverse operation in which the accepted value of the dissociation constant for carbonic acid is taken, and the value of δ is calculated, has been carried out by **Parsons**(19) from the results of electrometric observations made by himself and various others. It is obvious from what has been said that the value so deduced must be much smaller than that deduced from conductivity measurements, and as a matter of fact, **Parsons** found that it worked out to about ·50 for ·02 m. $NaHCO_3$ solution at 38° (instead of about ·85 as found by the conductivity method by **Walker** and **Cormack**). **Michaelis** and **Rona**(21) fix the values of k and δ in blood at $4 \cdot 4 \times 10^{-7}$ and 0·605 respectively. Now, as shown by **L. J. Henderson**, the H-ion concentration of a bicarbonate solution is given by the equation

$$[H]^{\cdot} = \frac{k[CO_2]}{\delta[NaHCO_3]},$$

and, although a more comprehensive formula has been worked out by **Parsons**, this may be taken as substantially correct for the purposes of the present argument. It appears then, that the results of hydrogen electrode determinations can only be made to agree with this if we either greatly increase the accepted values of k or greatly reduce those of δ.

It is especially interesting to see what happens if one takes for the calculation the values for k and for δ determined by such an independent method as the conductivity method used by **Walker** and **Cormack**. The result of such a calculation in the case of ·02 m. $NaHCO_3$ is that the curve falls almost exactly on the colorimetric curve which I have worked out. The values derived from this curve are given below, and the calculated curve is shown in Fig. 4. For this calculation δ was taken as ·851 and k as $3 \cdot 04 \times 10^{-7}$, since the observations were made at 20° C.

The values of δ shown in the fourth column are (roughly) calculated by the reverse process, assuming the observed $p.H$ figures as correct and taking $3 \cdot 04 \times 10^{-7}$ as the value for k. The high figure 1·19 in the first line is doubtless due to hydrolysis, and the steadily falling values throughout the remainder may be attributed to the same cause. When

TABLE IV. Calculated and observed $p.H$ and values of δ for bicarbonate solutions.

For ·02 m. $NaHCO_3$ solution				For ·02 m. $NaHCO_3$ + ·18 m. NaCl solution		
$p.CO_2$ mm.	$p.H$ calc.	$p.H$ obs.	δ calc.	$p.CO_2$	$p.H$ obs.	δ calc.
10	8·04	8·14	1·19	10	7·84	·54
20	7·73	7·76	·93	20	7·56	·57
30	7·56	7·58	·92	30	7·38	·57
40	7·43	7·45	·90	40	7·26	·57
50	7·34	7·34	·86	50	7·16	·57
60	7·26	7·24	·84	60	7·08	·57
70	7·19	7·18	·84			
80	7·13	—	—			

values for δ in the solution containing NaCl ·18 m. are calculated as in the seventh column, this is seen to be about ·57 and not subject to the same regular diminution with rising CO_2 tension which is shown by the plain aqueous solution of bicarbonate. **Parsons**(19) has calculated the mean value of δ for this solution at 38° C. from the results of **Milroy** (*l.c.*), and, assuming the accepted value for k, finds that it is about ·36: as in the other cases given, this indicates that the electrometric method gives too high results for hydrogen ion concentration.

Application of the Results.

The production of formic acid by catalysis in the hydrogen electrode method probably accounts for the importance of using caustic alkali quite free from carbonate, and water free from carbon dioxide, in the preparation of standard phosphate solutions for the indicator method; the presence of carbonate or carbon dioxide in small amount, while not greatly affecting the true hydrogen ion concentration in such a heavily buffered solution, would cause an electrometric determination to be too far on the acid side when the solution was standardised. If phosphate solutions containing carbonate and standardised by the hydrogen electrode were used in determining the reaction of blood, it is hardly necessary to point out that the results obtained would be in agreement with those obtained by the hydrogen electrode. The occurrence of this catalysis suggests that others may occur and explain other abnormal results with the hydrogen electrode.

It is important to recognise that in blood (and probably also in sea-water) there is a constant difference between the electrometric and colorimetric results, and I have given reasons for believing that this is due to an error in the former method. If absolute values are required, a correction should be applied to the hydrogen electrode determinations by adding 0·2 to the observed $p.H$. The reaction of human arterial blood for instance, is about 7·36 according to the hydrogen electrode, whereas that of a number of samples drawn by arterial puncture was found by Ross(22), using the colorimetric method, to be in the neighbourhood of p.H 7·6, and my own (venous) blood when brought to 40 mm. CO_2 has a p.H of 7·55. If comparative results only are required, either method may be used, provided the results of the two methods are not compared with one another.

Another important application concerns the calculation of the reaction of the blood from determinations of the free and combined carbonic acid of the plasma. This convenient and rapid method, the development of which we owe especially to the valuable researches of Hasselbalch, gives results which agree with those obtained by the hydrogen electrode. This agreement is on account of the fact that the value of the constant $p.K_1$ is itself obtained from electrometric observations, and not from values of k and δ determined by independent methods. It would be easy to redetermine values for $p.K_1$ which depended on those for k and δ derived from conductivity or other independent methods, and the hydrogen ion concentrations calculated from determinations of free and combined carbonic acid by the use of such a constant would then agree with those found by the colorimetric method. The indicator method could indeed be used for the determination of values of δ or k, which would obviously also lead to the same result. Another method of applying the necessary correction to the results of the Hasselbalch calculation would be to use the same formula and the same values of $p.K_1$ as he gives, and then to add 0·2 to the result, as in the following examples:

Plasma drawn from human blood at 38° C. and 41·6 mm. CO_2 had an alkali reserve of 63·3 p.c. $p.K_1$ is = 6·367, whence from Hasselbalch's formula p.H = 7·39. Adding 0·2 we get 7·59; the value found colorimetrically was 7·54.

In another case, for whole blood, the p.H according to the Hasselbalch formula was 7·115; adding 0·2 = 7·315; colorimetric result = 7·32.

Since such calculations involve the determination of the alkali reserve, which should for accuracy be that of the true plasma, as indicated by Poulton and Joffe(23), and also necessitate a knowledge of the carbon dioxide tension, it would seem to be more simple and direct to determine the p.H directly by the colorimetric method.

I wish to take this opportunity of expressing my gratitude to my colleague Dr H. H. Dale for the advice and for the many valuable suggestions which he has so kindly given in the course of this work.

Part of the apparatus used in this investigation was purchased out of a grant made by the Government Grants Committee of the Royal Society.

SUMMARY AND CONCLUSIONS.

1. Electrometric determinations of the reaction of blood or bicarbonate solutions yield results which represent H-ion concentrations about 60 p.c. higher (*p*.H 0·2 lower) than those given by the colorimetric method described by **Dale** and **Evans**.

2. The calculated reaction of a bicarbonate-CO_2 solution agrees with that determined colorimetrically, if values for the apparent dissociation constant of carbonic acid (k) and for the degree of ionisation of the bicarbonate (δ) as determined by the conductivity method are employed.

3. When constants (k or δ) derived from hydrogen electrode measurements are used in calculating the reaction of a bicarbonate solution, the results naturally agree with those of direct observation by means of the hydrogen electrode, because in such a calculation k is larger or δ smaller than is found by the conductivity method. Such calculated results obviously do not agree with colorimetric determinations.

4. It is inferred that it is the hydrogen electrode determinations, and not the colorimetric ones which are erroneous in these cases.

5. A source of error in the hydrogen electrode is indicated in that formic acid is produced by catalysis when the solution in the hydrogen electrode contains carbonates.

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THE ACTION OF IONS UPON THE FROG'S HEART.

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The following experiments were undertaken to analyse the effect upon the mechanical and electrical responses of the frog's heart of changes in the concentration of the kations, which are normally present in Ringer's fluid. The effect of such changes upon the mechanical response of the frog's heart was analysed by one of us (A. J. C.) in a previous paper(1), and the effect of certain of these changes upon both the mechanical and electrical responses of the frog's heart was studied by Mines(5).

Description of experiments. The experiments were commenced in the summer using *R. esculenta* but were repeated in the autumn on *R. temporaria*. The hearts were perfused, and the records of the mechanical and electrical responses were obtained, in the manner described by Mines(5). The string tension of the galvanometer was kept constant during each experiment. The Ringer's fluid used had a composition of NaCl ·65 p.c., $CaCl_2$ (anhyd.) ·012 p.c., KCl ·016 p.c., $NaHCO_3$ ·016 p.c.: the *p*.H (P_H) was 7·6. When *R. esc.* were used in summer the $CaCl_2$ content was raised to ·018 p.c. or ·024 p.c. Except for the fact that *R. esc.* required this additional calcium in summer to produce an optimal response, the hearts of the two species of frogs responded to changes in the ionic content of the Ringer's fluid in exactly the same manner. The hearts were allowed to beat at their natural rhythm in the earlier experiments, but since alteration of frequency was found to produce alterations in all of the factors measured, we maintained a constant frequency in the later experiments by arresting the heart with a Stannius ligature, and stimulating the auricle with break induction shocks.

The following factors were measured: (1) frequency (length of cycle), (2) *P-R* interval, (3) duration of electrical response (*DER*), (4) duration of mechanical response (*DMR*), (5) height of *R* wave (*HER*), (6) height of mechanical response (*HMR*), (7) duration of the rise of the *R* spike (Rise of spike). Of these factors the length of the *P-R* interval indicates the rate of conduction from auricle to ventricle, and the duration of the rise of the *R* spike was shown by Burdon Sanderson to be inversely proportional to the rate of intra-ventricular conduction. The significance of alterations in the height and duration of the electrical response will be discussed later.

The measurement of all these factors necessitated the taking of records at varying speeds; the rate of fall of plate varied from 1 to 10 cm. per second. The time was marked in fifths and twenty-fifths of seconds by a phonic wheel kept at a constant rate by an electro-magnetic tuning fork. The string used in the galvanometer had a resistance of 6000 ohms, and a deflection time of not more than ·02 sec., and was calibrated so that one millivolt caused a deflection of 1·5 cm. Measurements of length are expressed throughout in seconds, except the duration of the rise of spike which is expressed in twenty-fifths of seconds (σ = ·04 sec.). Measurements of heights are expressed in millimetres.

The effect upon the heart of isolation and perfusion. The mechanical and electrical responses of the frog's heart were found to be nearly the same after partial isolation and perfusion with Ringer as they were in the heart *in situ* with a normal circulation.

TABLE I.

	Temp.	Length of cycle	*P-R*	*DER*	*DMR*	Rise of spike in σ
R. temp. heart *in situ*	18°	1·7	·38	0·84	0·85	1·0
R. esc. heart perfused	18°	1·7	·42	0·92	1·12	0·9
R. temp. heart perfused. Stannius ligature stimulated	18°	1·9	·36	1·04	1·00	1·0

The effect of changes in the ionic content upon frequency. Table II shows the changes in the frequency of the sinus produced by the various changes in ionic content of Ringer's fluid that we examined. The results agree with those obtained by other workers. Dale and Thacker (2) found that a slight increase in alkalinity produced an increase in frequency but that acid and stronger alkali both caused a decrease in frequency. Sakai (8 & 9) found that reduction of NaCl to ·1 p.c., and increase of $CaCl_2$ to ·019 p.c., both caused a decrease in frequency, and that increasing the KCl from ·01 to ·02 p.c. caused no alteration. Our results agree with those quoted above, we found however that increase of KCl to ·064 p.c. produced a slight decrease in frequency, this change

TABLE II.

	Average alterations in frequency per min.			
Change made	Original frequency	After 5 min.	After 10 min.	Subsequent alterations
(1) NaCl reduced to ·32 p.c. Cane sugar added (5 exps.).	37	34	33	Heart continued regular beat for an indefinite period
(2) NaCl reduced to ·16 p.c. Cane sugar added (4 exps.)	41	33	29	Heart block (2 to 1) usually appeared after 30 min. Beats ceased in 1 to 2 hours
(3) KCl reduced to ·004 p.c. (4 exps.)	35	31	30	Heart block (2 to 1) after 20 min. Beats ceased after about an hour
(4) $CaCl_2$ increased to ·048 p.c. (5 exps.)	33	29	28	Heart block (2 to 1) after 10 min. Beats continued for more than an hour
(5) KCl increased to ·046 p.c. (4 exps.)	25	24	23	Arrest in diastole after 10 to 20 min.
(6) $CaCl_2$ decreased to ·003 p.c. (4 exps.)	28	28	29	Arrest in diastole after about 30 min.
(7) Fluid made acid (*p*.H 6·5) (4 exps.)	30	29	28	Arrest in diastole after about 30 min.
(8) Fluid made alkaline (*p*.H 9·5) (10 exps.)	29	32	83	Heart continued regular beat for indefinite period
(9) Fluid made alkaline (*p*.H 10·5) (2 exps.)	39	37	—	After a few minutes irregular rhythm. Arrest in systole after 20 min.

produced a great impairment of all the other functions of the heart and the slight effect produced upon the sinus is remarkable. Martin(4) showed that in the terrapin's heart the auricle was less affected by excess of potassium than was the ventricle. Mines(5) found that a decrease of calcium caused a marked increase in sinus frequency; we, however, found that this change produced no certain alteration in the frequency.

In general our results show that the concentration of ions normally present in Ringer is nearly the optimum for the activity of the sinus, and that the sinus frequency is less affected by changes in the ionic concentrations than is any other function of the heart.

The effect of alterations of frequency upon the electrocardiogram. Mines(6) showed that an increase in frequency caused the following changes in the response of the frog's heart, a decrease in the force of contraction, a decrease in the rate of conduction from auricle to ventricle and in the intraventricular conduction, and a great shortening in the duration of the electrical response of the ventricle. We confirmed these results as is shown by the following experiment.

The prolongation in the rise of spike, which we observed when the frequency was increased, was quite definite, but was much less than the

Exp. 1. *R. temp.* 15° C. Heart set up 3 hours previously.

Length of cycle	*P-R*	Rise of spike in σ	*HER*	*DER*
2·40	0·54	1·05	5·5	1·25
1·56	0·64	1·20	15·0	1·00
1·10	1·04	1·40	15·0	0·60

variation described by Mines. This difference is probably due to our string tension being greater than that employed by Mines; moreover we used a standard deflection in all cases, whereas Mines adjusted the tension of his string so as to obtain an easily readable auricular variation.

In order to avoid these secondary changes due to alterations in frequency we performed the majority of our experiments upon hearts in which the natural stimulus was abolished by a Stannius ligature and in which a constant frequency was maintained by artificial stimulation.

The effect of reducing the concentration of NaCl. The effect of reducing the NaCl content of Ringer to one-half was to improve the general activity of the heart, and the heart continued to contract normally in this mixture for an indefinite period. When the NaCl content was reduced to a quarter the heart passed into a condition of semi-systole, but continued to respond to stimuli for over an hour: the irritability of the auricle was however decreased and stronger stimuli were required to produce responses. When a fluid containing no sodium chloride was perfused, the heart immediately contracted into systole, and after about 10 minutes ceased to respond to artificial stimulation. The effects of these changes upon the electrical and mechanical response of the heart are shown in Exps. 2, 3 and 4.

Exp. 2. *R. esc.* 15° C.

Time	Length of cycle	*P-R*	*DER*	*DMR*	*HMR*	*HER*	Rise of spike in σ
3.55	1·70	·42	0·92	1·08	13	6	0·7
4.7	Change to Ringer containing NaCl ·32 p.c.; cane sugar 3·5 p.c.						
4.8	1·76	·46	0·88	1·24	13	10	0·9
4.14	1·64	·42	0·96	1·24	19	14·5	1·05
4.23	1·8	·48	1·00	1·40	18	10	1·1
Exp. 3. *R. temp.* 17° C.							
11.10	2·8	·44	1·08	1·12	16	9	0·8
11.11	Change to Ringer containing NaCl ·16 p.c.; cane sugar 5·5 p.c.						
11.11.30	2·8	·50	1·2	1·4	22	35	1·0
11.13	aur. 2·8 vent. 5·6	·50	1·65	2·2	25	—	1·0
11.16	2·8	·50	1·6	2·0	22	40	1·0
11.22	2·8	·45	1·5	2·0	25	35	2·0
11.25	2·6	·45	1·1	1·6	25	40	2·3
11.27	2·6	·40	0·72	1·8	16	40	3·0
11.45	2·8	·50	0·56	1·8	15	42	3·0
11.46	Change to Normal Ringer						
11.50	2·9	·45	0·8	1·8	17	35	2·0
12.10	2·9	·40	1·04	1·12	6	10	1·0

Exp. 4. *R. temp.* 17° C.

Time	Length of cycle	*P-R*	*DER*	*DMR*	*HMR*	*HER*	Rise of spike in σ
12.12	2·9	·40	1·0	1·12	6	9	1·0
12.20	Change to Ringer containing no NaCl, and cane sugar 7·0 p.c.						
12.21	2·9	·42	0·6	1·2	10	40	2·5
12.30	2·8	·60	1·2	1·6	5	<100	10–15
12.35	Change to normal Ringer						
12.37	2·8	·48	1·06	1·2	8	7	1·5

These results show that a reduction of the NaCl concentration to one-half improves the force of contraction, whilst a reduction to one-quarter increases the amplitude of contraction but produces a condition of semi-systole. Auriculo-ventricular conduction is not markedly affected by the reduction of NaCl to one-quarter, but the rate of intra-ventricular conduction is reduced to one-third by this change: total removal of NaCl impairs a-v conduction in a few minutes but produces a far greater impairment of intra-ventricular conduction. All these changes produce a progressive increase of the duration of the mechanical response of the ventricle.

Fig. 1. Effect upon electrical response of lack of potassium (*R. temp.*). A. Heart perfused normal Ringer (KCl ·016 p.c.). Artificial stimulus every 2·5 sec. B. Heart perfused for 6 minutes with Ringer containing ·004 p.c. KCl. Heart responding to alternate stimuli. The time marker in this and subsequent tracings shows $\frac{1}{5}$ and $\frac{1}{75}$ secs.

The duration of the electrical response of the ventricle alters in a peculiar manner, reduction of NaCl to one-half prolongs the *DER* slightly, reduction of NaCl to one-quarter at first prolongs the *DER*, and later as semi-systole develops, the *T* wave suddenly becomes negative, and the *DER* begins to shorten, and finally becomes less than half the normal length (cf. Fig. 4): total removal of NaCl produces at once a negative *T* wave and a shortened *DER*; later the *DER* lengthens, but this lengthening is due to the great impairment of the intra-ventricular conduction, and is completely accounted for by the increase in the duration of the rise of spike.

The effect of reducing the concentration of KCl. The reduction of the KCl content of Ringer to one-quarter produces semi-systole in about 10 minutes, and a 2 : 1 a-v block usually appears, but the heart continues to respond regularly to stimuli for at least an hour, although usually it is necessary to increase the intensity of the stimulus. Complete removal of KCl produces a strong systolic effect in a few minutes, after about 20 minutes the heart relaxes into diastolic arrest if the stimuli are discontinued; after about 40 minutes the heart ceases to respond to all stimuli and relaxes into diastolic arrest. The effects of partial and complete removal of potassium are shown in Exps. 5–7 and in Fig. 1.

Exp. 5. *R. esc.* 19° C. Artificial rhythm.

Time	Length of cycle	*P-R*	*DER*	*DMR*	*HMR*	*HER*	Rise of spike in σ
12.30	1·52	·32	0·88	1·12	22	25	1·3
12.31	Ringer containing KCl ·004 p.c. perfused						
12.31.30	1·60	·36	1·20	1·26	12	24	1·8
12.33	aur. 1·60 vent. 3·20	·40	1·60	1·72	25	22	1·9
12.41	1·50	·50	1·40	1·40	40	60	1·9
12.43	Normal Ringer perfused						
1.40	1·64	·30	1·04	1·12	24	35	1·0
Exp. 6. *R. temp.* 17° C. Artificial rhythm.							
4.32	1·64	·38	0·72	0·88	13	30	1·0
4.37	Ringer containing no KCl perfused						
4.40	1·48	·72	1·56	1·56	15	37	2·5
4.46	aur. 1·48 vent. 2·96	·68	2·8	3·0	24	70	2·0
4.50	aur. 1·48 vent. 2·96	·92	2·96	2·4	26	41	2·5
After 4.50 the heart continued to respond irregularly to stimuli for about 20 minutes.							
Exp. 7. *R. esc.* 18° C. Natural rhythm.							
11.30	1·20	·56	0·80	0·96	10	6	0·8
11.35	Ringer containing no KCl perfused						
11.37	1·36	·60	1·44	1·36	14	9	1·4
11.40	aur. 1·82 vent. 3·64	·76	2·52	2·72	15	4	1·6
11.55	3·0 (Irregular rhythm)	·96	1·44	1·64	16	14	2·1
12.0	Heart in diastolic arrest, no spontaneous contractions of auricle or ventricle, sinus contracting every 1·8 sec.						

These experiments show that although decrease of potassium produces an initial augmentor effect upon the force of contraction, yet the rate of conduction, both from auricle to ventricle and intra-ventricular, is diminished; this diminution commences directly after the change is made and becomes progressively greater. The great prolongation of the duration of the mechanical and electrical response of the ventricles is the most striking effect of lack of potassium; in one experiment the *DER* which was 0·08 sec. at the commencement, increased to 2·20 sec. after perfusion for 30 min. with Ringer containing KCl ·004 p.c. When potassium free Ringer was perfused all these changes were accentuated.

The effect of increasing the concentration of $CaCl_2$. When the $CaCl_2$ content of Ringer was increased from ·012 p.c. to ·048 p.c. imperfect relaxation in diastole occurred in a few minutes, but the heart continued to respond regularly to stimulation for an indefinite period (cf. Exp. 8 and Fig. 2).

Exp. 8. *R. esc.* 17° C. Artificial rhythm.

Time	Length of cycle	*P-R*	*DER*	*DMR*	*HMR*	*HER*	Rise of spike in σ
2.42	2·52	·44	1·04	1·40	11	23	1·0
2.47	Ringer containing $CaCl_2$ ·048 p.c. perfused						
2.49	2·60	·46	1·46	1·90	22	40	1·5
2.57	2·62	·44	1·32	1·80	23	45	1·4
2.58	Normal Ringer perfused						
3.10	2·52	·40	1·04	1·40	10	22	1·0

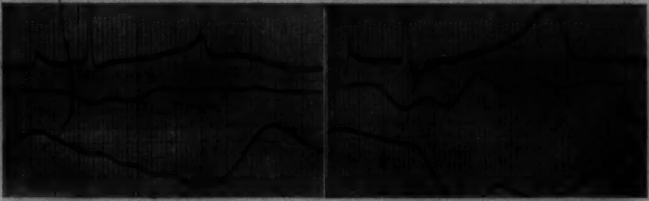

Fig. 2. Effect of excess of calcium. *R. esc.* Artificial stimulus. A. Normal Ringer ($CaCl_2$ ·018 p.c.). B. Heart perfused 10 minutes with Ringer containing $CaCl_2$ ·072 p.c. The curves show from above downwards, electrical response, movement of auricle, movement of ventricle, downstroke systole.

Exp. 8 shows that an increase of the $CaCl_2$ content to ·048 p.c. produces no effect upon the *P-R* interval, but that both the *DER* and the *DMR* are considerably prolonged and the intra-ventricular conduction is impaired. When the $CaCl_2$ content was increased to ·096 p.c., the NaCl content being reduced to ·55 p.c. to maintain isotonicity, after 10 minutes the *P-R* interval increased 30 p.c., and the duration of the rise of spike increased 100 p.c. The *DER* was at first increased; later, the *T* wave became negative and the *DER* shortened until finally a short diphasic response was obtained, like that obtained with lack of sodium.

The effect of increasing the concentration of KCl. When the KCl content of Ringer is increased from ·016 p.c. to ·064 p.c. the force of contraction is at once greatly reduced, the heart continues a feeble beat for a variable period, a-v block usually occurs, and finally the heart stops in diastole (cf. Exp. 9, Fig. 3).

Exp. 9. *R. temp.* 16° C. Artificial rhythm.

Time	Length of cycle	*P-R*	*DER*	*DMR*	*HMR*	*HER*	Rise of spike in σ
3.20	1·8	·36	1·04	1·00	12	25	1·0
3.22	Ringer containing KCl ·064 p.c. perfused						
3.22.30	1·4	·48	0·60	1·40	5	8	—
3.30	1·7	·8	1·08	1·6	4	3	8·0
3.31	Normal Ringer perfused						
3.40	1·7	·4	0·88	—	20	40	1·5

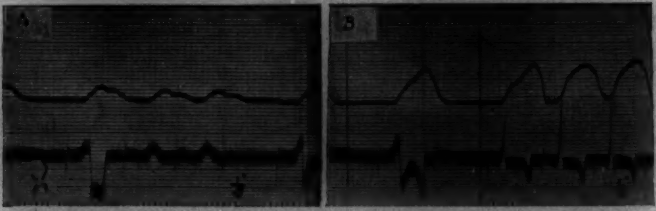

Fig. 3. Effect of excess of potassium. *R. temp.* Artificial stimulus. Heart perfused for 10 minutes with Ringer containing KCl ·064 p.c. At B, normal Ringer perfused. A and B are continuous. Upper curve ventricular movement, lower electrical response.

The effect of excess of potassium was studied in five experiments, and in all cases a great impairment occurred both in the force of contraction and in the conduction of the impulse both from auricle to ventricle and within the ventricle. In all cases the general shape of the electrical response became very abnormal (Fig. 3). These extensive changes in the electrical variation occurred at a time when the mechanical response was still visible.

The effect of diminishing the concentration of $CaCl_2$. The effect of reducing the calcium content of Ringer to a quarter ($CaCl_2$ ·003 p.c.) produces an effect upon the mechanical response very similar to that produced by an excess of potassium. When calcium free Ringer is perfused the heart is arrested in diastole in about 5 minutes (cf. Exps. 10–12).

Exp. 10. *R. esc.* 18° C. Artificial rhythm.

Time	Length of cycle	*P-R*	*DER*	*DMR*	*HMR*	*HER*	Rise of spike in σ
3.55	3·2	·44	0·96	—	—	8	0·8
3.56	Ringer containing ·003 p.c. $CaCl_2$ perfused						
3.57	3·2	·46	0·94	—	—	14	—
4.3	3·2	·46	0·92	—	—	18	0·9
4.7	3·1	·48	1·00	—	—	25	0·9
4.8	Normal Ringer perfused						
4.30	3·1	·44	1·08	—	—	8	0·8

Exp. 11. *R. esc.* 18° C. Artificial rhythm.

1.40	1·60	·34	1·00	1·12	24	35	1·0
1.51	Ringer containing $CaCl_2$ ·003 p.c. perfused						
1.52	1·56	·35	1·00	—	3	10	1·0
1.55	1·60	·42	0·92	—	2	8	1·0
1.56	Normal Ringer perfused						
2.10	1·68	·34	0·96	1·16	37	24	1·0

Exp. 12. *R. esc.* 18° C. Natural rhythm.

5.11	1·20	·34	1·0	1·0	35	—	1·0
5.12.30	Calcium free Ringer perfused						
5.13	1·64	—	1·08	1·12	6	—	0·9
5.20	1·56	—	1·12	—	1	—	1·1
5.40	All visible movements of auricle and ventricle ceased						
5.45	1·7–2·5	·44	1·18	—	—	—	1·0
5.46	Normal Ringer perfused						
5.47	1·32	·40	0·88	1·0	11	—	1·0

Mines(5) showed that after a heart had been arrested by lack of calcium a nearly normal electrical response was obtained: our results confirm this observation and show that arrest of the heart by lack of calcium produces no certain changes in the electrical response. It is true that the *P-R* interval and the *DER* are prolonged, but these changes are relatively slight, and it must be remembered that when the heart ceases to beat perfusion of fluid stops and as a result acid must accumulate in the heart, and this increase in acidity may easily account for the changes in the electrical response. By the use of indicators we found that the fluid inside the heart arrested by lack of calcium reached a p.H of 6·8 in about 5 minutes. Such alterations in p.H due to cessation of flow are however quite insufficient to account for the very extensive changes in the electrical response observed with excess of potassium.

The effect of increasing acidity.

Exp. 13. *R. esc.* 19° C. Artificial rhythm.

1.42	2·55	·40	1·12	1·32	15	29	0·9
1.46	Ringer perfused p.H 6·5						
1.46.30	2·60	·48	1·28	1·36	8	17	1·0
1.52	2·70	·56	1·28	1·34	6	15	1·1
1.53	Normal Ringer perfused						
2.20	2·60	·44	1·00	1·40	10	28	1·0

Exp. 14. *R. temp.* 17° C. Artificial rhythm.

Time	Length of cycle	*P-R*	*DER*	*DMR*	*HMR*	*HER*	Rise of spike in σ
4.32	2·96	·52	1·46	—	—	5	1·0
4.36	Ringer perfused *p*.H 6·5						
4.38	2·96	·72	1·68	—	—	4	1·25
4.46	2·96	1·0	isoelectric	—	—	4	1·25

The effect of a feebly acid Ringer is to reduce the force of heat greatly, and also to produce very great impairment of conduction from auricle to ventricle. The *DER* is increased slightly. These results agree with those of Mines(5). In addition our experiments show that the intra-ventricular conduction is not greatly impaired.

The effect of increased alkalinity. The effects of moderate alkalinity (*p*.H 9·0) are slight, the amplitude of beat is somewhat increased but the heart does not relax completely in diastole. Stronger alkalinity (*p*.H 10·5) produces a systolic effect after a few minutes (cf. Exps. 15 and 16).

Exp. 15. *R. temp.* 19° C. Artificial rhythm.

Time	Length of cycle	*P-R*	*DER*	*DMR*	*HMR*	*HER*	Rise of spike in σ
4.5	2·8	·28	1·06	0·96	13	9	1·0
4.12	Ringer perfused *p*.H 9·0						
4.13	2·8	·24	0·88	0·90	14	5	—
4.17	2·8	·26	0·88	0·92	13	8	0·9
4.40	2·76	·28	0·90	0·88	13	6	0·9
4.45	Normal Ringer perfused						
5.0	2·68	·36	1·12	0·88	10	3	1·0

Exp. 16. *R. temp.* 17° C. Artificial rhythm.

1.30	2·8	·50	1·00	1·1	8	11	1·6
1.35	Ringer perfused *p*.H 10·5						
1.36	2·8	·40	1·00	1·1	10	9	—
1.45	2·8	·42	0·80	1·28	8	30	1·5
2.15	2·8	·34	1·28	1·4	6	40	2·5

These results show that moderate alkalinity improves conduction from auricle to ventricle, the *DER* is definitely shortened and the *DMR* is not altered. Stronger alkali causes the heart to contract into systole but the electrical changes show surprisingly little alteration, for, even after the heart is in systole, the conduction from auricle to ventricle is more rapid than normal, although the intra-ventricular conduction is distinctly impaired.

DISCUSSION.

The effects produced by the various changes of ionic content are obviously of very great complexity. We are unable to put forward any theory to explain many of these changes. We have found no constant relation between the variations in the factors measured which held good for all, although relations often appeared that were common to two or three ionic changes.

We found (Exp. 2) that an increase of frequency impaired conduction from auricle to ventricle and within the ventricle, and caused a great shortening of the *DER*; these results agree with those obtained by Mines(6). De Boer(3) showed that premature beats in the frog's heart also showed these effects and also that in the premature beat the *T* wave was negative and the *HER* was usually increased; Exp. 2 agrees with this last observation. De Boer also showed that the alterations in the *HER* and the *T* wave could be accounted for by the diminished rate of conduction. Our experiments with changes in the ionic content however failed to show any regular connection between the rate of intra-ventricular conduction and the *HER* or the shape of the *T* wave. For with excess of KCl the rate of intra-ventricular conduction is greatly impaired but the *HER* is diminished (Exp. 9), and with excess of calcium the intra-ventricular conduction is slightly impaired but the *T* wave is strongly positive (Fig. 1).

A rise of temperature increases the rate of conduction from auricle to ventricle, increases the intra-ventricular conduction, and greatly shortens the *DER*.

Exp. 17. *R. temp.* Artificial rhythm.

Temp.	Length of cycle	*P-R*	*DER*	*HER*	Rise of spike in σ
15	1·8	·616	1·06	11	1·2
26	1·8	·374	0·70	4	0·9

The *HER* is diminished with a rise of temperature; this agrees with de Boer's hypothesis that an increase in the rate of intra-ventricular conduction tends to reduce the *HER*. It is interesting to note that both increase of frequency and increase of temperature cause a great shortening of the *DER*, although these two changes produce opposite effects upon the rate of intra-ventricular conduction; this fact indicates that the *DER* is not directly dependent upon the rate of conduction, and an inspection of the variations in the *DER* produced by ionic changes will show that there is no certain relation between the variations in the *DER* and the variations in any of the other factors measured.

A summary of the functional changes observed when various alterations are made in the ionic concentrations is shown in Table III. Column 1 was obtained from experiments in which the frequency was allowed to alter and the remaining figures are from experiments in which a constant frequency was maintained. These changes have been discussed in the course of the paper, but certain of them are of particular interest and require special consideration.

TABLE III. Approximate effects after 10 minutes of ionic changes upon the functions of the heart (changes expressed as percentage alteration of normal figures).

	Alterations produced						
Change in ionic concentration	Frequency (beats per minute)	Rate of conduction from auricle to ventricle (*P-R*)	Rate of intra-ventricular conduction (rise of spike)	Duration of electrical response (*DER*)	Height of mechanical response (*HMR*)	Duration of mechanical response (*DMR*)	Increased systolic tone (sys.) or increased diastolic relaxation (dias.)
(i) NaCl reduced to ·016 p.c.	−30	−10	−60	+50	+50	+90	sys.
(ii) KCl reduced to ·004 p.c.	−15	−30	−30	+40	+80	+25	sys.
(iii) $CaCl_2$ increased to ·048 p.c.	−15	0	−30	+30	+100	+30	sys.
(iv) KCl increased to ·064 p.c.	− 8	−60	−80	−10	−60	*	dias.
(v) $CaCl_2$ reduced to ·003 p.c.	+ 3	−20	0	+ 4	−90	*	dias.
(vi) Increased alkalinity *p*.H 10·5	− 5	+20	−30	−20	0	+15	sys.
(vii) Acidity *p*.H 6·5	− 7	−30	0	+10	−60	*	dias.

* The impairment of the mechanical response in these cases makes the accurate measurement of the *DMR* impossible.

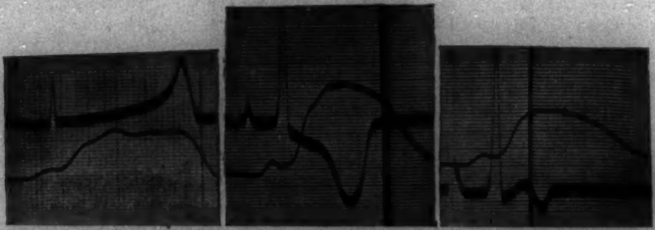

Fig. 4. Effect of lack of sodium. *R. temp.* Artificial stimulus. A. Normal Ringer. B. After perfusion for 14 min. with Ringer containing NaCl ·16 p.c. with 5·3 p.c. cane sugar added. C. 2 minutes after B.

When the NaCl is reduced to one-quarter and the fluid is kept isotonic with cane sugar the a-v conduction is not altered; the intraventricular conduction however is greatly impaired, and the *DER* shows a remarkable series of changes; it is at first increased, and later shows a great shortening (cf. Fig. 4). When a sodium free fluid is perfused the a-v conduction is impaired and the *DER* is shortened immediately. In both cases the shortened *DER* is associated with a negative *T* wave. These changes associated with lack of sodium show a remarkable resemblance to the effects produced by strophanthin.

Mines and Clark(7) showed that strophanthin caused in the perfused heart of the frog an impairment of a-v conduction, an increase in the duration of the mechanical response and an initial increase in the *DER* which was followed by a slow decrease. De Boer(8) using a frog's heart *in situ*, with natural circulation found that digitalis and antiarin caused a slight impairment of a-v conduction, and a great impairment in intra-ventricular conduction, and increase in the *HER*, and, in the final stages of poisoning, a markedly negative *T* wave. We tested the effect of strophanthin upon the isolated heart beating with a constant artificial frequency, and obtained results essentially the same as those described above.

Exp. 18. *R. temp.* 17° C. Artificial rhythm.

Time	Length of cycle	*P-R*	*DER*	*DMR*	*HMR*	*HER*	Rise of spike in σ
3.14	2·36	·54	0·96	1·20	15	9	1·0
3.15	Amorphous strophanthin ·0002 p.c. perfused						
3.24	2·36	·54	1·36	1·56	25	8	1·0
3 26	2·36	·56	0·72	1·00	16	5	1·0
3.30	2·36	·58	0·40	1·12	10	20	1·5*
3.40	2·36	·94	0·36	0·80	2	30	2·2†

* Imperfect diast. relax. † Systolic contr.

A comparison between the effects produced by strophanthin and those produced by lack of sodium (Exp. 3), shows that the two effects are remarkably similar, in both cases there is a prolongation of the *P-R*, an initial increase of the *DER* and the *DMR* followed by a great shortening of the *DER* which is accompanied by a change in the sign of the *T* wave which becomes negative; this shortening of the *DER* is not accompanied by any great shortening of the *DMR*; finally in both cases the intra-ventricular conduction is greatly impaired, and this impairment is accompanied by a great increase in the *HER*.

Somewhat similar effects to those described above can be produced by reducing the KCl or by increasing the $CaCl_2$, but in these cases the resemblance to the action of strophanthin is not so striking. The systolic effect produced by alkali however shows changes in the electrical response quite different from those described above.

The action of potassium and calcium upon the mechanical response of the heart suggests that these two ions have exactly antagonistic effects, for opposite changes in their concentrations produce almost identical effects. The effects produced upon the electrical variations of the heart suggest however that the antagonism in this case is much less exact, for lack of calcium will cause complete arrest of the movements of the heart without producing any certain change in the electrocardiogram, while excess of potassium produces great alterations in the electrical response before it arrests the movements of the heart. This suggests that although excess of potassium and lack of calcium produce an apparently similar effect upon the mechanical response, yet the former acts by interfering with conduction of the wave of excitation, whilst the latter acts by abolishing the power of the muscle to respond to the wave of excitation. Lack of potassium and large excess of calcium have on the whole a similar action on the electrical response. The

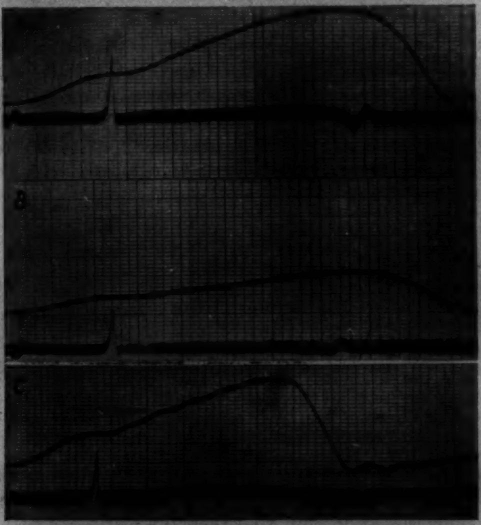

Fig. 5. Antagonism of calcium and potassium. *R. temp.* Artificial stimulus. A. Normal Ringer KCl ·016 p.c. $CaCl_2$ ·012 p.c. B. Ringer containing KCl ·016 p.c. and $CaCl_2$ ·003 p.c. C. Ringer containing KCl ·004 p.c. and $CaCl_2$ ·003 p.c.

relation between the actions of these two ions is however more complicated than these statements would suggest, for the effects produced on the electrical response by an alteration in the concentration of one ion can be in part antagonised by a compensatory alteration in the concentration of the other ion. The reduction of the calcium content to one-quarter causes a great impairment of the mechanical response, but if the potassium content is also reduced to one-quarter the mechanical response returns to normal as is shown in Fig. 5. On the other hand the disturbance in conduction produced by excess of potassium can be temporarily removed by an increase in the calcium content as is shown in Fig. 6. This effect is however only a temporary one and no excess of calcium will permanently antagonise the impairment of conduction produced by excess of potassium.

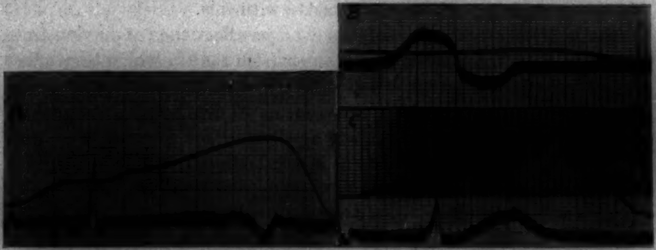

Fig. 6. Antagonism of calcium and potassium. *R. temp.* Artificial stimulus. A. Normal Ringer (KCl ·016 p.c., $CaCl_2$ ·012 p.c.). B. After perfusion with Ringer containing KCl ·064 p.c., $CaCl_2$ ·012 p.c. C. 1 minute after perfusion with Ringer containing KCl ·064 p.c. and $CaCl_2$ ·048 p.c. The improvement in the electrical conduction shown in C only lasted for 5 minutes, but the improvement in the mechanical response continued for 15 minutes.

Any alteration in the relative concentration of calcium and potassium produces an immediate effect upon the mechanical response of the heart but if the relative concentrations are kept constant the absolute concentrations can be varied within wide limits without disturbing the mechanical response. Any alteration in the relative concentrations also produces a marked effect upon conduction, but the limits within which the potassium content can be varied and a normal conduction be obtained by a compensatory alteration in the calcium content are comparatively narrow.

All changes in ionic concentration if sufficiently extensive will, as is known, in time impair all the functions of the heart, but there are marked differences in the rate and intensity of the changes in the different functions. In the first place the optimal ionic content of Ringer is different for different regions of the heart. **Dale and Thacker**(2) showed that the optimal C_H for the initiation of spontaneous contractions was lower for the ventricle than the auricle and that, when a sufficiently alkaline solution was perfused, the ventricle became the pacemaker for the heart. **Sakai**(9) studied the effect of changes in ionic content upon the isolated ventricle of the frog, and found that the frequency of spontaneous contractions was increased by reduction of NaCl (to ·1 p.c.), of KCl (to ·005 p.c.), or increase of $CaCl_2$ (to ·0325 p.c.), whereas he found that the frequency of the sinus was reduced by lack of Na or excess of Ca, and our results agree with this.

Table III shows that most ionic changes affect the conduction from auricle to ventricle in a different manner from that in which they affect the intra-ventricular conduction. Alterations in the potassium content produce similar changes in the two forms of conduction, but lack of sodium, excess of calcium and alkali, all of which produce a systolic effect, greatly impair intra-ventricular conduction, but have little effect in impairing a-v conduction; whereas lack of calcium and acid, which produce a diastolic effect, impair the a-v conduction, but do not impair the intra-ventricular conduction.

The changes in the conduction of the electrical impulse in the ventricle show no close relation to the changes in the height of the mechanical response of the ventricle; this point is most clearly shown by the effects of lack of calcium.

The changes in the shape of the *T* wave are so inconstant that we are unable to draw any certain conclusions concerning them. It is however interesting to note that the general appearance of the short diphasic response obtained in the later stages of sodium lack shows a resemblance to the response obtained from skeletal muscle.

CONCLUSIONS.

1. Alterations in the concentration of the ions normally present in Ringer's fluid, if sufficiently extensive, will, as is known, impair all the functions of all parts of the heart, but a moderate degree of change affects the different regions of the heart to an unequal extent, and also acts with different intensity upon the different functions of the ventricle.

2. Alterations in the potassium content produce a greater impairment of the conduction of the electrical variation, both from auricle

to ventricle and within the ventricle, than any other ionic change studied.

3. Reduction of the calcium content has little effect upon the conduction of the electrical variation.

4. Potassium and calcium act as true antagonists as regards their effect upon the muscular response, but they act as antagonists only to a very limited extent as regards their action on the conduction of the electrical variation.

5. The effect of lack of sodium shows a striking resemblance to the effect of strophanthin.

6. Lack of potassium or sodium, and excess of calcium all produce increase of systolic tone in the heart and produce rather similar variations in the electric response; increased alkalinity also produces increased systolic tone, but produces markedly different effects upon the electrical response.

7. Alterations in the *p*.H affect the electrical response much less than do alterations in the potassium content.

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The expenses of this research were defrayed by a grant from the British Medical Association.

THE IMMEDIATE ACTION OF VOLATILE SUBSTANCES. By W. E. DIXON and FRED RANSOM.

(From the Pharmacological Laboratory, Cambridge.)

The object of this paper is to point out that the immediate physiological effects produced by certain volatile substances are most readily explained by their physical properties. Two types of experiments have been performed: (*a*) on the bronchioles, (*b*) perfusions of isolated organs.

The bronchiolar experiments were made on cats which were killed by pithing: artificial respiration was carried on by a pump delivering a constant volume of warm air at each thrust. Blood-pressure under these conditions varies from 50 to 80 mm. of mercury. The condition of the bronchioles was gauged by measuring the volume of the middle lobe of the right lung and the blood-pressure was recorded from the left carotid artery. The arrangement of the experiments was similar to those we have already described (1). It has been pointed out (2) that it is easy to obtain reflexes from the nose causing contraction of the bronchioles. In these experiments every care was taken to prevent vapours reaching the nose, but to ensure that no impulses passed down the vagi these nerves were severed. A great many irritant substances in very small quantities when inspired cause broncho-constriction. Thus ammonia gas, chlorine and bromine vapour exert this effect. The principal feature of this action is the long latent period, generally 15 to 30 seconds, and generally the gradual onset of the bronchial constriction. A typical effect is shown in Fig. 1. Here is seen the long latent period but the onset of constriction is more sudden than usual. The rise of blood-pressure to the right of the tracing is asphyxial. Effects such as these are produced by many irritant substances and generally traces only are sufficient to produce the effect.

With such irritants we are not directly concerned, but with volatile substances which possess little irritant properties and which are relatively non-poisonous: chloroform will serve as an example. If 0·35 c.c. of chloroform is injected into the rubber tube fixed to the tracheal tube, then under the conditions obtaining in the experiment about one-third reaches the lungs, the rest escaping by the orifice on the side of the metal tracheal tube. Such an inhalation causes an immediate constriction of the bronchioles lasting some ten or twelve seconds, the maximal contraction is reached immediately and recovery is more gradual: the effect can be produced any number of times without diminishing the degree of action, provided a sufficient time is given for the chloroform to disappear (Fig. 2). The effect only occurs at the beginning of the inhalation and only if the administration of the chloroform is moderately sudden, and it may be prevented by giving the drug very gradually. The effect passes off in under half a minute whether the administration of chloroform is continued or not.

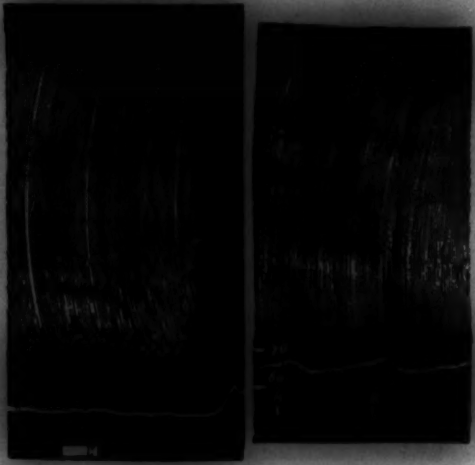

Fig. 1. Fig. 2.

Cat. Lung vol. Blood-pressure.

Fig. 1. Vagi cut. Inhalation of ammonia vapour by the tracheal tube. Duration of tracing 72 secs.

Fig. 2. Inhalation of 0·25 c.c. chloroform at *A*, and a second inhalation of the same strength at *B*. Time in seconds.

The constriction is so sudden that it suggests some local or axon reflex, but it is not possible to adopt this view since the effect is quite as well defined when the vagi are paralysed by atropine. It is more difficult to believe that the effect can be directly on the muscle for many reasons. The absence of latent period is against this and also the transient nature of the contraction; further, plain muscle does not respond in this way to poisons but rather as a gradual and prolonged contraction. Moreover, some of the volatile drugs which produce this effect have a specific action on plain muscle in the opposite direction. Thus amyl nitrite relaxes all plain muscle throughout the body, yet a sudden inhalation of this drug may induce a sudden broncho-constriction like chloroform, and when the dose has been large may even constrict the bronchioles to exclude the passage of air (Fig. 3). Another possible explanation is that the vapour causes sudden turgescence of the mucous membrane, but the mucous membrane of the bronchioles in the cat cannot swell up in this way, and the effect can be obtained on the cadaver. We are forced then to reject the view that the effect is due to a chemical reaction between the drug and tissue and search for some physical explanation of the phenomenon.

Fig. 3. Cat. Lung vol. The bronchioles were in an irritable condition owing to the previous administration of 1 mgm. physostigmine. After the broncho-constriction the strength of the pump was insufficient to dilate the bronchioles. Time in seconds.

Straub(3) suggested that the inhibition of the heart by muscarine is caused by the physical process of the passage of muscarine through the limiting layer of the cell and that when it has passed this layer it cannot cause inhibition, and he showed that in the Selachian heart this explanation would account for the facts. Supposing that the passage of the chloroform through the membrane, or the difference of the chloroform tension on the two sides of the epithelial cells lining the bronchioles, resulted in such a broncho-constriction, then one would expect to obtain a more prolonged effect if the circulation were cut off. To test this the animal was bled to death, the artificial respiration being continued unchanged. When all movements of the heart had ceased, a dose of chloroform was given under exactly similar conditions as before (Fig. 4). The bronchioles contract immediately but the contraction, which reaches its maximum at once, is perhaps a little more profound than before, and much more prolonged, often extending to a minute. Recovery is very gradual. The effect may be produced several times if a sufficient interval is allowed between each inhalation. This experiment excludes the interpretation that the effect may be due to turgescence of the mucous membrane. Other substances which produce this effect are amyl nitrite, alcohol, ether (very slightly), petroleum ether and several others.

A second series of experiments was made by perfusing isolated surviving organs of cats and dogs. The animals were anæsthetised and bled to the utmost limit from one carotid. Some were killed first by pithing and then bled: this method has the advantage that the blood and tissues are free from anæsthetic, a matter of no little importance in these experiments. The defibrinated blood filtered through glass-wool served as the perfusing fluid. Experiments were made with the hind limbs, lungs and intestines. The limbs and lungs were perfused by the method described by Brodie and Dixon which enables a graphic record to be taken of the outflow from the vein. The perfused tissue was kept in an oven at body temperature. During lung perfusions sometimes the lungs were partly distended and sometimes artificial respiration was kept up with warm air.

Fig. 4.

Fig. 5.

Fig. 4. Cat. Dead. Lung vol. This is the same animal as in the experiment shown in Fig. 2. Inhalation 0·25 c.c. chloroform.

Fig. 5. Perfusion of hind limbs of cat. Injection of 0·2 c.c. alcohol in normal saline. At each *x* 6 c.c. of blood was removed. Duration of tracing 4½ mins.

When the perfusion is running smoothly the thrust of the pump, whose work it is to remove the blood from the venous to the arterial reservoir, is regulated to keep pace with the venous flow so that the quantity of blood in the venous reservoir remains constant. The perfusion pressure varied; for the limbs and intestines 75 to 85 mm. mercury were sufficient, and for the lungs from 20 to 35 mm. Considerable care is necessary in the administration of the drugs. Any little increase in pressure increases the flow; this difficulty was avoided by injecting the drug between the arterial receiver and a glass-wool filter interposed between the receiver and the artery. The injection of salt solution or Ringer into such an artificial circulation by diminishing the viscosity of the blood increases the flow; to avoid this effect the drug should be dissolved in blood. In some of the experiments described the drugs were nevertheless for convenience dissolved in Ringer, but in these instances the activity of the drug caused vaso-constriction in spite of the diminished viscosity.

Fig. 6.

Fig. 7.

Fig. 6. Perfusion of cat's lung. Injection of 6 c.c. chloroform (1 in 200) in normal saline. Duration of tracing 5 mins. 10 secs.

Fig. 7. Perfusion of cat's limbs. Injection of 1 c.c. of a saturated solution of amyl nitrite. At the height of vaso-constriction 8 c.c. of blood was added to the venous receptacle. Duration of tracing 6 mins.

If a small dose of alcohol such as 0·1 c.c. is made up to 1 c.c. in Ringer and injected into the arterial system of an artificially perfused organ, there is a diminution of outflow from the vein immediately the alcohol reaches the organ. The diminished outflow lasts only about 30 seconds and is succeeded by some increase in flow. The effect can be obtained in the dog and cat, and in all the organs that were investigated.

Second and third injections produce a similar action. Fig. 5 shows the type of effect in a normal manner.

Chloroform produces an effect of the same nature but more intense. 2 or 3 c.c. of 1 in 200 chloroform dissolved either in blood or Ringer and injected into the arterial system of the perfused organs of a cat or dog diminishes the outflow from the vein. This diminution begins at once but lasts only about half a minute when the blood flow returns to its normal rate or for a short time may be a little quicker (Fig. 6). The effects may be produced many times by successive injections but tend to become less marked; and a time may come when an injection is without effect.

If chloroform is given by inhalation through the tracheal tube during artificial respiration the effect is less decided. In the cat under these conditions the rate of outflow from the pulmonary vein diminishes almost immediately and the vaso-constriction is slightly more prolonged than when the injection is made directly into the circulation (Fig. 8). In the dog the effect is less decided, but some vaso-constriction is here also the rule. The interpretation of the experiments in which chloroform is absorbed through the lungs is not so simple as when the chloroform is injected into the circulation because the first effect is broncho-constriction. Still, as we have shown elsewhere, moderate broncho-constriction does not interfere with the pulmonary circulation. It therefore appears that if our interpretation of the mode of action is valid, it is of no moment on which side of the vessel wall the drug obtains access; so long as there is a partition coefficient on the two sides, a tendency to constriction is present.

Fig. 8. Perfusion of cat's lung during artificial respiration with warm air. Administration by inhalation ½ c.c. chloroform. Duration of tracing 3½ mins.

With amyl nitrite it is not easy to obtain constant results since it is one of the most powerful vaso-dilators. In first injections vaso-constriction was generally very definite and was followed by very marked dilatation. Fig. 7 shows the effect on the limb of a cat: there is a very profound constriction followed by a most abrupt dilatation.

These experiments show that isolated blood vessels exhibit the same type of response to the drugs under consideration as the bronchioles.

As with the bronchioles chloroform, alcohol, amyl nitrite and to a minor degree ether, under the conditions given, produce vaso-constriction which begins at once and lasts for 30 seconds; this is generally followed by dilatation, profound with amyl nitrite, less with alcohol, and little or none with chloroform. These vaso-constrictor effects do not represent the specific action of the drug, nor would a specific effect begin and end abruptly in this fashion. Nor can the effect be due to the introduction of saline solution or Ringer into the fluid because this always tends towards dilatation: a 2 p.c. or stronger solution of sodium chloride invariably causes dilatation. A 1 or 2 p.c. solution of urea in Ringer, a substance which diffuses very readily into all tissue cells, produces initial vaso-constriction in both lungs and limbs: the effect is not profound, it lasts longer than the constriction by volatile substances but still passes off rapidly. For these reasons we believe that this initial action must be ascribed to the passage of the substance through the vessels. As soon as a balance is obtained the effect ceases, and each successive injection tends to produce the same or a slightly diminished effect. It will be noticed that the contraction time for plain muscle in the bronchioles and vessels is about the same. It does not seem important which way the drug passes since inhalation of chloroform from the lungs produces the same type of temporary vaso-constriction.

Fig. 9.

Fig. 10.

Fig. 9. Cat. Urethane. Blood-pressure. Inhalation of amyl nitrite through a tracheal tube. Time in secs.

Fig. 10. Cat. Urethane. Blood-pressure. Inhalation of chloroform through a tracheal tube. Time in secs.

It must be well recognised in animal experiments that inhalation of chloroform and amyl nitrite often causes at first a short rise of blood-pressure, before the characteristic fall due to the specific action of the drug on the heart and blood vessels respectively. Nevertheless we are unaware of any references to the condition and append two tracings taken haphazard, which illustrate the condition (Figs. 9 and 10). The rise in blood-pressure immediately following on inhalation through the tracheal tube we think is due to the action we have described.

CONCLUSIONS.

The first action of alcohol, chloroform, ether, amyl nitrite, petroleum ether and many other volatile substances administered by inhalation is to cause broncho-constriction lasting about 30 seconds. The same substances injected into the perfusing fluid of surviving organs, artificially perfused, cause vaso-constriction lasting about 30 seconds. Reasons are given for believing that this action is due to physical processes.

The expenses of this Research were defrayed by a grant from the Royal Society.

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STUDIES IN THE REGENERATION OF DENERVATED MAMMALIAN MUSCLE. IV. Effects of Massage and Electrical Treatment in Secondary Sutures[1]. BY F. A. HARTMAN AND W. E. BLATZ.

(Conducted by the Research Committee Medical Services, Department of Militia and Defence, Ottawa, Canada, in co-operation with the Physiological Laboratory of the University of Toronto, Canada.)

WE have previously reported the results of massage and electrical treatment (1) in cases of primary suture. A large number of the war cases are secondary sutures. Therefore in the present research we have studied the treatment in delayed or secondary suturing. The animals have with few exceptions been treated and tested as before described (1).

Operation. At the primary operation, the tibial nerves were severed under aseptic conditions, the cut ends being separated in order to check regeneration of the proximal end until the desired time. This was done by one of the following methods:

(1) The proximal end was pulled by means of a ligature into a sterile rubber tube, which was then either doubled back and tied to muscle or extended toward the knee beyond the distal end.

(2) The proximal end without a tube was reversed and sewn to an adjoining muscle.

(3) The proximal end was left intact while the distal end was turned aside from the former and sewn to the sheath of the gluteus muscle.

(4) The proximal end was left untouched, but the distal end was covered by a sheath of Cargile membrane which was then stitched to adjoining muscle.

Method (1) was employed in 26 of the electrical series and 36 of the massage series. Method (2) was used in four of the massage series. Fourteen of the electrical series were treated according to method (3).

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[1] Approved for publication by the Acting Director-General of Medical Services, Department of Militia and Defence, Ottawa, Canada.

Twenty-three of the electrical series and 19 of the massage series were treated according to method (4).

The denervated legs were protected by padded aluminium boots(3). After a lapse of from one to six months a secondary operation was performed. The tube or Cargile membrane was removed. The old ends of the nerve were freed from adhesions and freshened by clipping away the ends. Then by means of a silk suture the two ends were brought as close together as possible. When a gap occurred it was never more than 10 to 12 mm.

Treatment. In both electrical and massage series the right limb was treated, the left serving as a control. Each animal was treated daily, except Sundays.

The electrical treatment consisted of a slow surging galvanic current produced by a McIntosh No. 4 polysine generator, plate and point electrodes as described in the preceding investigation were applied to the limb so as to cause contraction of the gastrocnemius group. The hair was closely clipped and the skin soaked with 0·8 p.c. NaCl solution. Two kinds of treatment were used: (*a*) a current which elicited a contraction just above the minimal, 30 contractions to the minute being produced for four minutes; (*b*) a current which elicited a "moderate" contraction forty times each minute for five minutes. The currents which produce these results range from 20 to 30 volts and 1 to 20 milliamperes.

All animals which were massaged were subjected to a light stroking and kneading from the ankle to the knee for eight minutes.

Passive movements, for not more than two minutes, were given to right and left limbs at each treatment in both "massage" and "electrical" series.

Testing. The method of testing was exactly the same as in the preceding research(1). This consists essentially of a series of contractions of the gastrocnemii groups against loads, these contractions being elicited by galvanic make shocks while the rabbit is under the influence of ether. The tests were begun within two or three days after the primary operation and continued at intervals of 10 to 14 days until the completion of the experiment. Rabbits were used exclusively in this study.

RESULTS AND DISCUSSION.

No distinction could be made between the "electrical" and "massage" series except in regard to loss of weight (*vide infra*). Therefore the two series will be considered together in most instances.

Condition of animals. The general condition of the animals as well as the local condition of the denervated part was watched carefully. The former was indicated by frequent weighing, appetite, and by the healthy appearance. Occasionally an animal died apparently from pneumonia, brought on by cooling too soon after anæsthesia.

We lost more than 50 animals out of 125, on account of a fractured tibia. This was a very large increase over the losses from the same cause in the preceding research. In the first place the direct cause is the metal boot together with the frequent strain occasioned by the awkward position which the denervated limb is permitted to assume by the animal. A new type of boot which was later abandoned for the old type, undoubtedly accounts for some of the fractures although by no means all of them. The practice of permitting the animals to recover from anæsthesia by placing a number of them together on a canvas cot was no doubt responsible in a number of instances. It was done so that the body heat would be mutually beneficial.

There was a tendency however for those animals which recovered first to get on top of the others. This would add pressure to the boot-encased limbs and might cause a fracture. Still another reason why more fractures should occur in this research is the much greater duration of the interval during which the hind limb was helpless because of the prevention of nerve regeneration. We thought that there might have been a chemical change in the bone, rendering it more easily fractured, but analyses of the relative amount of animal and mineral matter showed little change from normal and no appreciable difference from the analysis of the humerus in the same animal. Although the bones of but three rabbits were analysed, the results were so uniform that we are inclined to discredit a changed composition sufficient to alter the strength of the bone. A number of experiments had to be abandoned because of the development of stiff or infected heels. In rare cases an animal was lost from anæsthesia.

Fifty rabbits out of 125 were successfully carried through the secondary operation and from one to nine months beyond.

Of 111 animals whose weight was followed after the primary operation, 46 gained, 55 lost and 10 neither gained nor lost in weight. This is based upon the weight at the termination of each experiment. The gain might be as high as 30 p.c. and the loss as low as 30 p.c. In one instance it reached the unusual decrease of 50 p.c. Sometimes there would be a slightly fluctuating decrease following the primary operation. Again the primary decrease might be succeeded by an increase which would be reversed by the secondary operation. Loss of weight as a criterion indicated poorer health of the rabbits treated by electricity, for 63 p.c. of those lost weight as compared with 34 p.c. which lost in the case of massage.

Right-footedness. Of the 125 rabbits used in this series 76 or 60·8 p.c. possessed stronger muscles on the right side (gastrocnemius group). The rest were stronger on the left side. This is not in accord with our previous researches. In the first, 53 p.c. were left-footed, 28 p.c. were right-footed and the rest were doubtful (60 animals). In the second, 63 p.c. were left-footed, 32 p.c. were right-footed and the remainder doubtful (43 animals).

A compilation of all three researches gives the following:

	Right stronger	Left stronger	Doubtful
Research I ...	17	32	11
„ II ...	14	27	2
„ III ...	76	49	0
	107	108	13

These figures leave us in doubt as to whether rabbits are typically stronger on one side than on the other.

Faradic stimulation. It is well known that faradic stimulation is much less effective than galvanic stimulation in denervated muscle. However we attempted to compare the responses, of the right and left muscle groups, to faradic stimulation. We did this in 73 animals of this series. The response in all cases was so much smaller than that to galvanic stimulation that it was useless as a test. We made these tests at various stages in degeneration.

Nerve regeneration. We shall describe briefly the condition of the nerve at the time of the secondary operation.

In the rubber tube series the proximal end of the nerve which had been pulled into sterile rubber tubing at the primary operation was found either just barely encased by the end of the tube or merely plugging the entrance. When encased by the tube the end of the nerve was necrotic for a greater length. The tube was often partially filled with a lymph-like fluid. Sometimes a nerve bulb was formed at the entrance of the tube in the case of plugging. In only three instances had the proximal end entirely escaped and reached the distal end of the nerve by a few fibres. Rarely did the proximal nerve ever appear to be growing down the tube, then it seemed to be embedded in a lymph clot within the tube. In a great majority of cases therefore the rubber tube not only kept the proximal end of the nerve from reaching the distal end but it checked the growth. The manner in which the nerve had slipped to a large extent out of the tube suggested that the rubber was repellent to growth perhaps on account of some chemical substance in the rubber. The distal end was always found more or less held down by fibrosed adhesions which had to be loosened before suturing.

The method of leaving the proximal end of the nerve intact and turning the distal end aside and then suturing to the sheath of the gluteus muscle, was not successful. In 11 out of 14 experiments some of the proximal fibres had found the distal end at the time of the secondary operation. In some instances the proximal end had sent out a fan-like growth which appeared to be an attempt to find the distal path. This method merely delayed the linking of the regenerating end with the distal path.

The method of covering the distal end of nerve with a sheath of Cargile membrane sewn to the adjoining muscle prevented union with the proximal end in all cases except one. This method left the proximal end free but it did not seem to regenerate so rapidly as in the case when the proximal and distal ends are joined by suture. Although this was not proven it was indicated in a number of cases where two months after the primary operation the proximal end was found to be retracted toward the notch. In two instances, however, the proximal end had sent out a fan-like growth of fibres. The distal end of the nerve was found encased in a fibrous mass which had to be dissected away before suturing.

Using as evidence an increase in the muscular response to galvanic stimulation 14 of our animals showed complete regeneration of some of the nerve fibres. The increase in five of these was slight. Had the animals been continued longer, the recovery would undoubtedly have been more complete.

At the time of discontinuing the series, regeneration was tested in 11 animals by faradic stimulation of the exposed sciatic and inspection of the exposed gastrocnemius. Nine of these animals showed regeneration by this method. All of these were three months or more past the secondary operation. In ten additional animals the union of proximal and peripheral portions of the nerve was merely examined. Nine of these appeared to be well united.

The influence of treatment. Graphs were plotted in every experiment as in the previous research (1). From these the question of the influence of treatment was studied. In all of those animals which never reached the secondary operation no benefit could be shown from either massage or electrical treatment.

Of those rabbits continued one month or more beyond the secondary operation two in the electrical and two in the massage series showed positive improvement on the treated side. (See Table.) Three animals in the electrical and three in the massage series showed slight improvement on the treated side. On the other hand one in the electrical and four in the massage series became worse on the treated side. The balance showed little difference.

TABLE OF EXPERIMENTS.

Electrical treatment				Massage			
Number of exps. made	Months between primary and secondary operation	Months observed after second. operation	Benefit from treatment	Number of exps. made	Months between primary and second. operation	Months observed after second. operation	Benefit from treatment
2	2	1	None	1	2	1	None
1	2	2	Slight	2	3	1	"
4	2	3	None	2	1	2	"
1	2	3	Slight	1	3	2	"
1	2	3	Positive	1	4	2	"
1	3	3	None	9	2	3	"
2	3	4	"	2	2	3	Slight
1	3	4	Slight	2	2	3	Positive
2	4	4	None	1	3	3	None
1	4	4	Positive	1	7	3	"
1	5	5	None	2	5	4	"
				1	7	4	"
				2	4	5	"
				1	7	5	"
				2	3	8	"
				1	3	8	Slight
				1	2	9	None

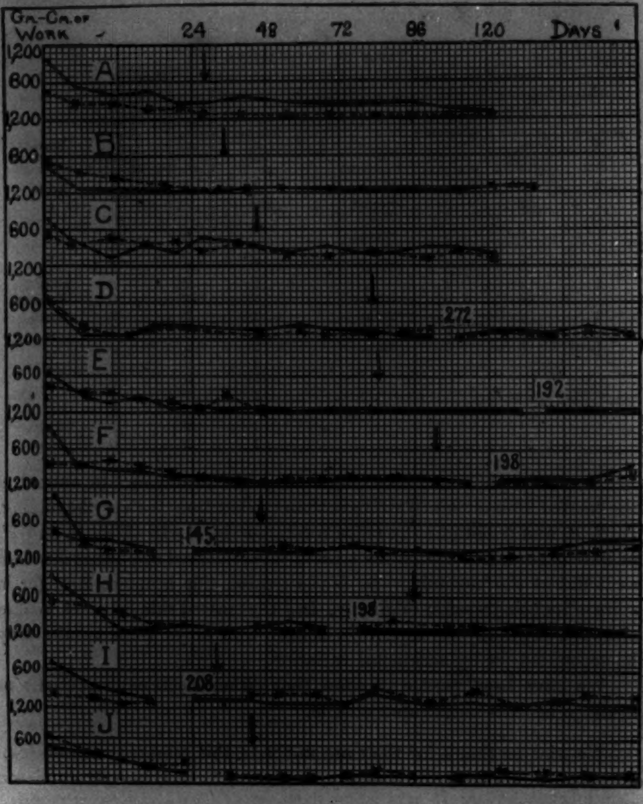
The accompanying Chart contains typical examples. In animal *A* the treated limb remained stronger than the left from the very beginning. In *B*, *E*, and *G* both finally reach a common level. In *C*, *F*, *G*, and *H*, in spite of some fluctuation the treated side remained somewhat stronger as it did at the beginning. In *D* the treated side becomes stronger in spite of its being weaker originally. In *I* the treated side becomes weaker although originally stronger. We believe that all of these results may be accounted for by accidental variation.

We have been unable to show that either electrical treatment or massage benefits denervated muscle.

We wish to thank for their assistance in this research: Misses Joan Campbell, J. Halliday, K. Halliday, D. Hearn, N. R. Hearn, E. Jamieson and A. Kent.

SUMMARY.

1. The ends of the severed tibial nerves were separated in order to check regeneration for periods of time ranging from one to seven months. At the end of this period the two ends were freshened and brought together by suture.



Comparative strength of the denervated gastrocnemius groups. Solid line indicates treated muscle; broken line control. Arrow indicates time of secondary suture. B, E, F, G treated by slowly surging galvanic current, the others treated by massage.

2. The power of the gastrocnemii groups of the two sides was tested by galvanic stimulation immediately after denervation and at frequent intervals until the conclusion of the experiment.

3. The gastrocnemius muscle group on the right side was treated almost daily by either a slow surging galvanic current or by massage.

4. Of 125 rabbits started in this research 60·8 p.c. possessed stronger muscles on the right side (gastrocnemius group) the remainder being stronger on the left side.

5. Neither massage nor electrical treatment appeared to benefit the denervated muscle.

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ON THE FIBRILLATION OF THE HEART.

By S. de BOER.

(From the Pathological Laboratory, Amsterdam.)

Various theories have been advanced on the origin of fibrillation, of which the following may be mentioned: Kronecker(1) assumed the existence of a nervous centre coordinating the ventricular systoles. Fibrillation, he said, was caused by paralysis of this centre. Trendelenburg(2) considered that fibrillation was due to very rapid stimuli causing different muscular regions to contract at different rates. Winterberg(3) in 1907 thought that a number of foci of contraction were formed in a sector of the heart muscle, and that the multiple stimuli sent out by them led to dissociation of the systole. This theory was supported by Rotberger and Winterberg(4). Rihl, Hering and Laevis succeeded in making the ventricle of the mammalian heart fibrillate through the influence of a combined vagus-accelerans-stimulation. They also established a certain relation between extra systoles and delirium cordis. Subsequently they abandoned their original theory. In their later experiments with the aid of the string galvanometer, and of the differential electrodes of Clement, they established

(1) That the oscillation frequency of the auricles during fibrillation amounted to 3000–5000 a minute and that of the ventricles to 800–900 per minute. In some cases they observed equal deflections in a regular rhythm.

(2) That by stimulation of the vagus flutter (flattern) of the auricles could be changed into fibrillation (flimmern). A considerable shortening of the refractory stage was considered by them as the final cause of fibrillation.

(3) Up to a certain frequency the number of the suspension deflections was equal to that of the electric curves.

On the basis of these data they looked upon delirium cordis as a simple tachysystole. Various objections may be raised against this theory. It is sufficient to mention the improbability of complete systoles occurring at the rate of 3000–5000 a minute.

The researches of A. G. Mayer(5), who cut a ring from the bell of the large medusa Cassiopeia, are important. In such rings he established a local block and by stimulating on one side he could set going a contraction wave, which travelled in one direction only. When he now removed the block before the circuit was completed by the wave, the contraction wave continues to circulate round the ring during many hours.

Mines(6) cut out rings from the heart of the tortoise and from the auricles of elasmobranch fish, and stimulating at one spot obtained a contraction which passed repeatedly round and round the ring. There was a "circulating excitation" producing a "circulating rhythm." The conditions necessary for the production of the rhythm were, he said, those produced by rapid stimulation, viz. a slowing of the propagation of the wave of contraction, a lessening of the duration of contraction, and a decrease of the refractory period. Different circulation excitations produced in this way in different parts of a heart chamber he considered to be the cause of fibrillation. The example given of "reciprocating rhythm" is the passage of contraction from auricle to ventricle, back from ventricle to auricle and so on, in consequence of a difference in the refractory period of two parts of the A.-V. bundle.

Garrey(7) independently of Mines' experiments, cut rings from the fibrillating ventricles of turtles and found that the fibrillation continued if the rings were broad, but ceased and gave way to regular contraction passing round and round the rings when the rings were made narrow. The regular contraction he called "circus contraction." He noticed that the contraction passed sometimes by one part of the ring and sometimes by another. He considered that the essential conditions of fibrillation was the occurrence of "blocks," *i.e.* of portions of decreased conductivity and excitability occurring now in one part and now in another. In the normal heart the excitation wave reached the heart at various points and the parts of decreased excitability only responded when the excitation wave reached them after a circuitous course. Levy(8) gave a general support to Mines' theory.

I began my researches[1] on this subject after I had been occupied for many years in studying the general physiology of the frog's heart. In this animal fibrillation has been examined only by means of mechanical registration. Gewin's researches in this direction are well known.

The string galvanometer which rendered great service in experiments on fibrillation in mammals (**Winterberg** and **Rotberger**) could not be utilised for the frog's heart because fibrillation could be excited only by faradic stimulation and induction currents interfered with the electric curves. It is, however, essential to study the action currents in the fibrillating frog's heart, since the results are more reliable than those obtained with the heart of the mammal. In fact the experimental data gathered from the frog's heart (**Engelmann**, **Gaskell**) have been corroborated by data obtained from the mammalian heart (**Langendorff**, **Hering**, **Winterberg** and **Rotberger**), and from man by clinicians (**Wenckebach**, **Mackenzie**).

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[1] The following researches and the new theory on fibrillation of a heart chamber were communicated by me in 1919 and in the beginning of 1920 (9).

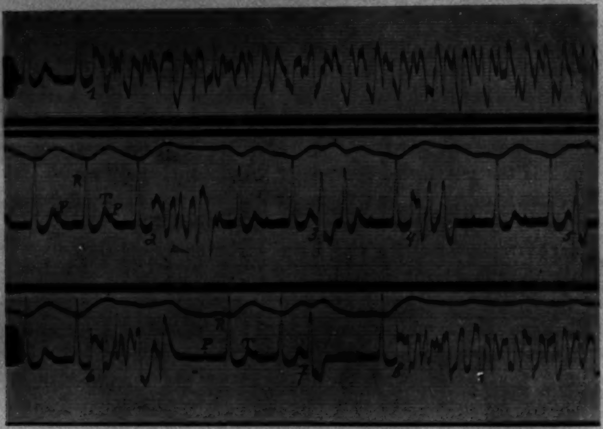

Fig. 1.

It was essential, therefore, to excite fibrillation in the frog's heart, without making use of faradic stimulation. It had struck me as early as 1914 that a single induction shock applied to the ventricle repeatedly engendered fibrillation in it. Later in the course of my alternation-experiments with the bled frog's heart through extra stimulation, I noticed the phenomenon so frequently that I investigated it more closely. It appeared that fibrillation of the ventricle occurred after a single induction-shock only when this was applied directly after the close of the refractory stage which always accompanies the systole immediately preceding. This is clearly illustrated by the curves of Fig. 1, which were registered[1] half an hour after the bleeding of a suspended frog's heart. In the upper row of curves an induction shock was given to the base of the ventricle at 1, a short time after the close of the refractory stage. Fibrillation of the ventricle was the result, which manifested itself in the string curve by totally differing deflections whose rhythm was very irregular. Similar results were achieved at 2, 6 and 8.

It will be seen that the post-undulatory pause, after fibrillation excited in 6, may be lacking (after 2). After 3 an extra systole is interpolated by the extra stimulus, a phenomenon that may occur with a slow heart-beat, as was first shown by Trendelenburg. In this case the stimulus was administered at a much later period of the ventricle (towards the close of the *T*-deflection) so that a fully co-ordinated extra systole was the result. In the same way a complete extra systole is generated at 7, because here also the stimulus was applied later. Not a single exception to this did I find in a large number of experiments with more than 100 frogs. While fibrillation of the ventricle could be generated by a single induction shock at the very com-

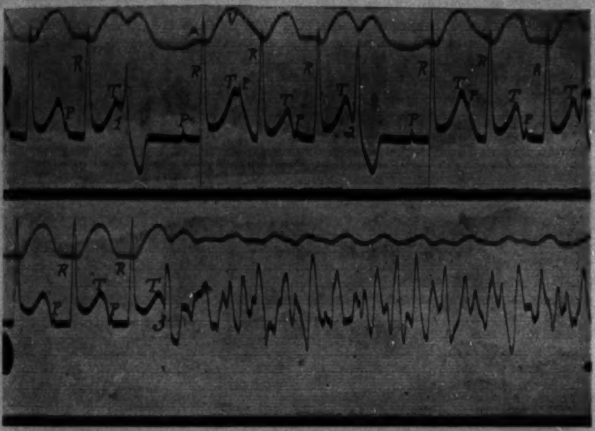

Fig. 2.

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[1] In this and the following registrations one non-polarisable electrode was placed on the auricles and one on the apex of the ventricle. The tension of the string was in all experiments such that 1 m.v. yielded a deflection of $1\frac{1}{2}$ mm.

mencement of the excitable period, I invariably obtained a fully co-ordinated extra systole, when the stimulus was applied at a later stage of the excitable period with the same force and at the same spot.

To the brief delirium, originating at 4 after the extra stimulus I shall revert lower down. Fig. 2 shows that during an experiment the metabolic condition of the ventricular muscle must deteriorate considerably before delirium can be brought about. In the upper row a stimulus was twice administered to the base of the ventricle 15 minutes after the bleeding (at 1 in the beginning, at 2 in the middle of the descending branch of the *T* deflection). In both cases a complete extra systole of the ventricle appeared. The second row of curves was registered a quarter of an hour after the bleeding, and at 3 a little before the middle of the descending branch of the *T* deflection an extra stimulus was given to the base of the ventricle. Although the stimulus was applied later than at 1, ventricular fibrillation followed, *i.e.* the general condition of the ventricular muscle having grown worse, the stimulus to the ventricle must be applied later, in order to generate an extra systole. It is obvious that the electric deflections are again very irregular. Shortly after this registration, this fibrillation stopped spontaneously. When a few

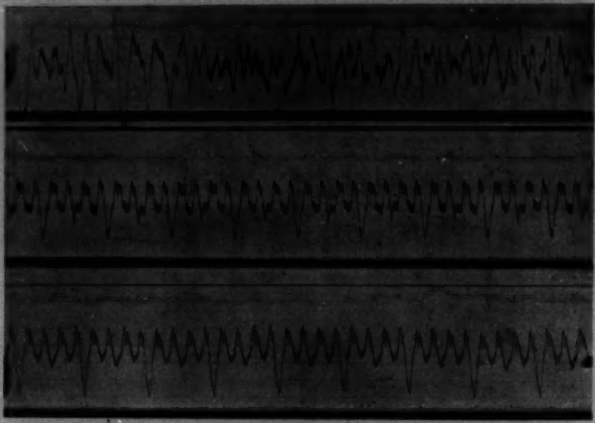

Fig. 3.

minutes afterwards I again applied a stimulus to the ventricle a permanent fibrillation of the ventricle ensued, which I registered for 1½ hours (see Fig. 3). The top curves were photographed five minutes after the beginning of the delirium. We see that now the deflections, as in Fig. 2, are irregular. The 2nd row was registered ½ hour after the commencement of the fibrillation. Here the curves present a totally different aspect. A certain regularity in the deflections can be observed. Three smaller deflections occur between each two larger ones, but the four successive deflections are all different and the rhythm is irregular. But these groups of four deflections recur continually. The bottom curves were taken 1½ hours after the commencement of the fibrillation. Though the deflections are slightly altered, the regularity of the delirium remains. In both registrations the two successive, equal deflections are at the same distance from each other. In the suspension curves the regular deflections of the auricular contractions may be observed (in the lower registration the suspension curve is slightly interfered with by a momentary movement of the frog). I noticed also by inspection that the auricles continued beating during the fibrillation.

I have the registration with the string galvanometer of two more frogs' hearts that kept fibrillating for longer than half-an-hour after a stimulus. There also the deflections of the string were initially irregular, and here also they became more regular afterwards. In these registrations large and small deflections alternated and the auricles continued beating regularly.

Significance of the results for an explanation of ventricular fibrillation.

I may first summarise the main results of this investigation. It should be remembered that the ventricle can only be made to fibrillate by an extra stimulus if this stimulus is applied directly after the close of the refractory stage. Fibrillation never appears when the stimulus is applied later with the same force and at the same spot; if it is, it will result in an ordinary extra systole. To these facts I attach great importance. The ventricle begins to fibrillate after an extra stimulus only when its metabolic condition is bad. This condition is still bad at the beginning of the excitable period, because in the short time after the termination of the preceding systole the ventricular muscle has not been able to recover. From this bad metabolic condition it follows:

(1) That the contractility of the ventricular muscle is bad. After an extra stimulus there is a brief small contraction. **This brief extra contraction is accompanied by a brief refractory stage.**

(2) That the conductivity of the excitation through the ventricle is slight.

These two circumstances are conclusive for the origin of the delirium. The conditions are quite different when the stimulus is applied at a later period. Then the metabolic condition is much better, because after the preceding systole the ventricle has had more time for recovery. Consequently the contractility and the conductivity are much better; then the excitation passes rapidly through the ventricle and a properly co-ordinated extra systole results from the stimulus.

In order to understand fully the origin of the delirium, we must first consider the brief delirium, since in some of our experiments the delirium was only of very short duration and consisted of two or three deflections in the mechanogram and in the electrogram. This is instanced in Fig. 1 (2nd row of curves at 4). Here we see after the electric stimulus three small deflections in the suspension curve with which electric deflections correspond. Now what does this mean? When looking at two or three deflections, we observe a phenomenon formerly described by me as deformed ventricular systoles, and which is known in the literature by the name of ventricular peristalsis. Similar deformed systoles also occur after digitalis poisoning[1].

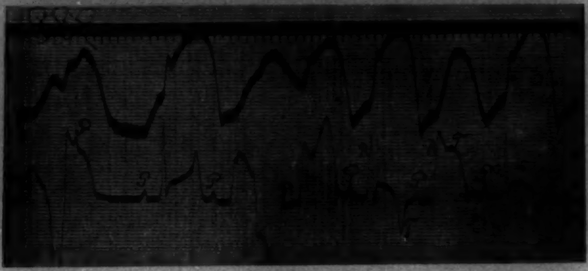

Fig. 4.

This is illustrated by the curves (Fig. 4) registered from a frog's heart 25 minutes after a subcutaneous injection in the thigh of 14 drops of digitalis dialysate. The first ventricular curve consists of two parts: first the suspension curve rises up to a certain point and at the beginning of the dilatation line a second rise begins. This form of the curve owes its origin to the circumstance that first a part of the ventricular muscle begins to contract; subsequently, owing to the bad metabolic condition the rest of the muscle comes into action with a prolonged latent stage; this causes a retarded contraction.

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[1] *Arch. Néerl. de Physiol.* 1. p. 530. 1917.

The electrogram registered at the same time fully confirms this statement. The third ventricular curve of the figure presents a break in the ascending branch and is, therefore, also deformed. During these deformed ventricular systoles the whole muscle is indeed made to contract, but in two or three rhythms. The same is the case with the brief delirium. After the extra stimulus which affects the ventricle at a moment when the recovery of the muscle is still unsatisfactory, part of the ventricle begins to contract. The proceeding excitation imparts contraction to the following portion only after a long lasting latent stage, so that the excitation passes through the ventricle in two stages. The brief delirium then is nothing but a deformed fractionated extra systole. Now upon this basis we can readily conceive the origin of the longer fibrillation in my experiments.

As set forth above, the refractory stage of the contraction, generated at the outset of the excitable period, is shortened. This shortening is of great moment for the lengthening of the delirium. When the excitation wave after an extra stimulus goes through the ventricle in stages, the time of such a circulation is lengthened considerably. And when the excitation wave reaches the starting point again, this contracts, because the short refractory stage of the preceding contraction has already come to a close. The excitation wave proceeds through the ventricle once more and again in jerks. Thus the excitation wave keeps on circulating through the ventricle, and fibrillation is checked only when it strikes on a refractory region. Then the post-undulatory pause sets in, which, however, may be absent (Fig. 1 after 2 in the second row).

After an extra systole, elicited later in the excitable period, the excitation wave does not begin a second course, because then it is checked by the refractory stage, which with this extra systole is of longer duration. The same relations exist with the normal rhythmic systoles. If in this case the refractory stage were absent or much shorter, the excitation wave would always continue its course in the closed muscular system of the ventricle which would not be able to pulsate rhythmically under the influence of the sinus impulses.

According to my theory, therefore, fibrillation of the heart is brought about by a non-co-ordinated contraction, not as Winterberg conceived this; viz. that sundry sources of contraction are functionating independently, but that the various regions of a ventricle contract successively and an excitation wave being once elicited may pass through a ventricle several times running; the ventricular delirium consists of a string of fractionated ventricular systoles. For fibrillation two conditions must be fulfilled: (1) the refractory stage must be shortened; (2) the conductivity of the stimulus through the ventricle must be decreased.

Winterberg and Rotberger believed that the only essential condition for the origin of fibrillation was a much shortened refractory stage. This is only true if also conductivity is bad.

The question arises, why after digitalis poisoning of the frog's heart deformed ventricular systoles are generated, but not fibrillation of the ventricle? This finds an explanation in the fact that after digitalis poisoning (in a toxic dosis[1]) the refractory stage of the ventricle is lengthened instead of shortened. Conductivity is then bad, so that a single deformed ventricular systole can arise, but the excitation wave cannot pass through the ventricle a second time.

But I cannot subscribe to the theory of Mines and Garrey that in fibrillation there are several circuits of different conductivity. On my theory the conduction is through one circuit, and in this the conductivity varies at several points so that the contraction proceeds in successive stages, *i.e.* in jerks.

In conclusion I may give a short account of how I arrived at my theory. In 1916 I made experiments on heart peristalsis after poisoning with digitalis (10), these I have alluded to above. Since the suspension curves and the electrograms of ventricle fibrillation of short duration were the same as those of the fractionated ventricle systoles after poisoning with digitalis, I concluded that during ventricle fibrillation as well as during ventricle peristalsis, the contraction wave was propagated through the ventricle slowly and in stages. In 1918 I made experiments on the velocity of propagation of the excitation through the ventricles during extra systoles with the string galvanometer (11). From these experiments I concluded that during the extra systole set up shortly after the end of the refractory period the excitation wave is propagated very slowly. It was known from the experimental work of Marey and Trendelenburg that the extra systole evoked after the refractory stage was small and had a brief refractory period. In consideration of these points I came to the conclusions I have set forth above.

[1] When speaking of digitalis poisoning in a toxic dosis, I mean a dosage that lengthens the refractory stage and retards conductivity.

In connection with this subject it should be mentioned that Wenckebach as early as 1907 described multiple systoles (Häufung d. Syst.) and advanced the theory that the first gave rise to the second and the second to the third. So far as I know this was the first suggestion that in a series of systoles each may arise from its predecessor.

SUMMARY.

In the preceding paper I have shown that if a bloodless heart of a frog is taken, a single electrical stimulus is capable of causing fibrillation of the ventricle. Thus a bad metabolic condition of the heart favours fibrillation. Essential factors in fibrillation, are as on Mines' theory, decrease in the rate of conduction and in the duration of the refractory period, so that a circulating excitation can be set up. A single stimulus in order to produce fibrillation in the bloodless heart must be applied directly after the refractory period. At this time the excitability of the muscle is slight, a slowly travelling contraction with a brief refractory period is produced, so that when the contraction completes its circuit, the muscle is again excitable and the contraction can spread again and again round the heart. The stimulus if applied later gives rise to a complete co-ordinated extra systole.

I conclude from the deflections recorded in the electrogram and suspension curves of heart peristalsis after poisoning with digitalis and of fibrillation of short duration that in fibrillation the excitation wave passes round the heart muscle in a series of stages each being marked by a deflection and that the *local* circulating excitation assumed by Mines and by Garrey do not exist. According to my theory fibrillation of the ventricle consists of a linking together of fractionated ventricular systoles.

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ON RECURRING EXTRA SYSTOLES AND THEIR RELATION TO FIBRILLATION. BY S. DE BOER.

(From the Pathological Laboratory, Amsterdam.)

IN the experiments I have dealt with in a preceding paper (1) I sometimes obtained in bloodless hearts of the frog, that is in hearts in a poor metabo-condition, a series of extra systoles on applying a single stimulus to the ventricle. The production of these as a result of a single stimulus gave me an opportunity of investigating their origin. Extra systoles in series have been called multiple systoles, and their production a 'Häufung' of systoles. The term 'multiple' suggests that extra systoles arise from different foci and it will be better to speak of them as recurring systoles.

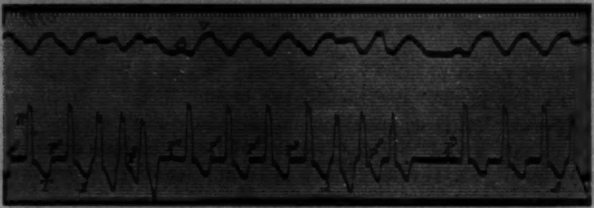

Fig. 1[1].

Figs. 1 to 3 are examples of records in which recurring systoles were obtained; we may first consider the relation of these to the time of applying the stimulus, and take some other features of the curves afterwards.

In Fig. 1 at 1 and at 2, a stimulus was applied to the base of the ventricle at the summit of the *T* deflection and in each case three extra systoles resulted. The suspension curve shows the relative height of the several contractions.

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[1] In every figure the electrograms were taken by placing a non-polarisable electrode at the apex and one on the auricles. The tension of the string was such that the interpolation of 1 m.v. caused a deflection of the string of 1⅓ mm. Time was registered in ⅕ second.

The curves of Fig. 2 are derived from the same frog's heart. At 1, an induction shock was applied to the base of the ventricle towards the close of the *T* deflection and gave rise to three extra systoles. After the induction-shock at 2 there was a mingling of extra systoles and fibrillation.

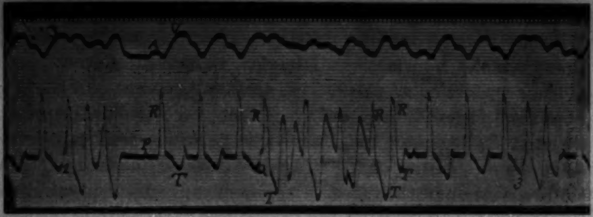

Fig. 2.

Fig. 3 shows the curves from the heart of another frog. At 1, stimulation at the base of the ventricle a little after the apex of the *T* deflection evoked four recurring extra systoles. At 2, another stimulus evoked five such extra systoles. But at 3, a stimulus was sent in a short time *after* the *T* deflection and the customary single extra systole only was produced.

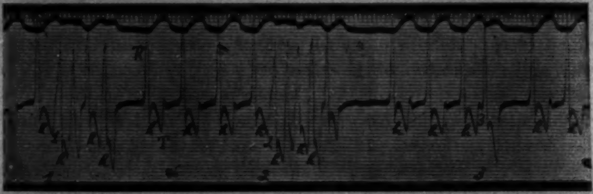

Fig. 3.

Thus in order to produce recurrent extra systoles, the ventricle must be stimulated at a certain short time after the end of the refractory period.

Now it is shown experimentally that as a stimulus is sent into a heart nearer and nearer to the end of the refractory period, the contraction wave is propagated more and more slowly (de Boer(3), Mines) and the refractory period accompanying the contraction is decreased in duration (Trendelenburg). As was pointed out by Mines for the ring experiments, when the decrease reaches a certain extent, the contraction wave on coming round to its starting point will continue its course, and a circulation rhythm be set up. We may, then, fairly conclude that recurrent extra systoles are circulating systoles, because each recurrent systole shows a complete electrogram with an *R* and a *T* deflection.

The curves given in Figs. 1–3 besides showing the occurrence of recurrent systoles from a single stimulus, show that they only occur when the propagation of the excitation wave is decreased. The *R* deflections are broadened, the *T* deflections have become more negative.

Burdon-Sanderson and Page(2) using an electrometer, pointed out that the rate of rise of the spike (*R* deflection) depends upon the rate of propagation of the wave. Mines confirmed this using the string galvanometer, and I have described the changes of the form of the record of the electrical change after slowing of the rate of propagation of the wave, so for extra systoles which were evoked near to the refractory period and at a later moment of the ventricle period(3). The changes are: a slower rate of rise and a broadening of the *R* deflection, an increase in depth of the *T* deflection, and of the line between *R* and *T*. As will be seen by reference to the figures, all of these changes occur to a greater or less extent in the electrograms of the recurrent systoles.

We may pass on to the consideration of the relation of the recurrent systoles to fibrillation. A connection between the two has been suggested by clinicians (Wenckebach, Lewis and others). The two have the common factors that in order to produce them, conduction must be slowed, and the duration of the refractory period reduced, so as to allow circulating contraction to occur. I have shown(1) that in the ventricle of the bloodless heart of a frog, fibrillation can be produced by a stimulus which is sent in *immediately* after the end of the systole. Thus in a ventricle in this state, whether a stimulus gives rise to a single extra systole, to recurrent systoles, or to fibrillation depends upon the time after the end of the refractory period at which the stimulus is sent into the ventricle.

Further evidence of the connection between recurrent systoles and fibrillation is shown by the fact that each may pass into the other. Levy ((4) p. 64) noticed that multiple ventricular systoles produced by adrenaline very frequently ended in fibrillation. Fig. 4 is an example of fibrillation ending in an extra systole. At 1, a stimulus was sent into the ventricle base a little past the summit of the *T* deflection

This induced ventricular fibrillation, but in a short time the fibrillation ceased, and there was an extra systole. (During the fibrillation the auricles continued beating regularly as shown in the slight elevation *A* in the suspension curve.) We must suppose that in this case the conductivity of the heart for some reason improves. I have often observed that fibrillation ends in a strong rise of the suspension curve, this is shown in the curves I have given elsewhere (1 and 5). This rise points to a contraction of a large area of heart muscle, and so to recovery of more normal excitability.

I wish to draw attention to one more point. Between the fibrillation and the extra systole a small negative deflection occurs (indicated by an arrow). It is impossible to say for certain how this deflection originated. It may be that after the fibrillation a retrograde excitation conduction has given rise to an auricular contraction, which at that moment coincides in the suspension curve with the extra systole curve. Then we should recognise a negative *P* deflection, since the excitation wave proceeded in a retrograde direction through the auricles. Secondly, it may be imagined that this negative deflection is generated by a bulbus contraction. We notice these bulbus electrograms during the ventricular electrograms of the periodic ventricular systoles and also during the electrograms of some 'gehäufte' extra systoles (indicated in the figure by *b*). Then the excitation wave would appear to have spread after the fibrillation over the bulbus arteriosus.

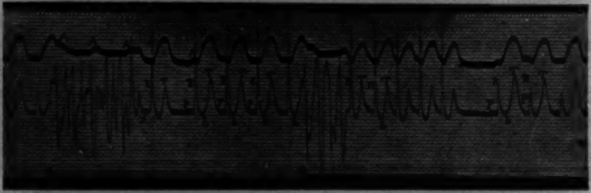

Fig. 4

At 2, the ventricular base is stimulated again a little past the summit of the *T* deflection. Again ventricular fibrillation is the consequence, while the auricles continue to pulsate in the undisturbed rhythm. After a short time, however, fibrillation passes into *five* recurring extra systoles, followed by a prolonged pause. In what manner this transition can occur is further examined by me and will be soon published.

The substance of this paper was communicated to the Royal Academy of Science of Amsterdam in March, 1920(6). Recently (August, 1920) Lewis(7) has published a theory of the flutter in the auricles of warm blooded animals of a very similar nature. It is satisfactory to find this close agreement of our views.

SUMMARY.

When a stimulus is applied to the ventricle of a bloodless frog's heart a short time after the end of the refractory period, a series of extra systoles may occur. But when the same stimulus is applied at a longer interval after the end of the refractory period to a heart capable of giving a series of extra systoles, one extra systole only is produced. It appears therefore that the phenomenon of recurring extra systoles (Häufung d. Syst.) only occurs when the heart muscle has not recovered its metabolic state. In this state the excitation wave is conducted more slowly, the refractory period is shorter, and the excitation is able to pass round and round the ventricle, *i.e.* a circulating contraction is set up.

Conduction through the muscle is slowed both in recurring extra systoles and in fibrillation; the difference between them is that in the former conduction takes place evenly, and in the latter it takes place in stages. The intimate connection of the two phenomena is shown by the fact that each may pass into the other.

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CAMBRIDGE: PRINTED BY J. B. PEACE, M.A., AT THE UNIVERSITY PRESS.

PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY,

May 15, 1920.

On the nature of the cells in the nerve plexuses of the iris. By J. N. LANGLEY.

In a recent paper Pollock(1) has drawn some general conclusions from previous experiments made by him on the iris. Since these conclusions are opposed to some I have drawn, I think it desirable to draw attention to the nature of the evidence offered by the original experiment(2).

The ciliary ganglion and the superior cervical ganglion were excised in rabbits and, after allowing an interval for degeneration, the nerves of the iris were stained with methylene blue. Some non-medullated fibres were found throughout the iris, and connected with them, angular cells and bipolar cells with several processes. Pollock considered that the cells were ganglion cells, and that some were on the course of sensory fibres, and some on the course of post-ganglionic motor fibres.

The inference to be drawn from the persistence of nerve fibres depends of course on whether all the post-ganglionic and sensory nerves were cut. The ciliary nerves were presumed to be cut, and accessory ciliary ganglia removed (as he mentions in his second paper), in the operation of cutting through the tissue round the optic nerve close to the eye. The success of the operation was not judged by post-mortem examination of the nerves at the sclerotic, but by the presence or absence of medullated fibres in the iris when stained with methylene blue. This method seems to me uncertain, partly because more or less of the medullated fibres are commonly unstained at the time the terminal plexus is well stained, and partly because the point of their course at which medullated fibres lose their medulla varies. Further in view of the great overlapping which occurs in the peripheral distribution of sensory nerves, it is not improbable that a few sensory fibres pass to the iris from the conjunctiva. Lastly there is the possibility of a few aberrant nerve cells being present in particular cases in the rami of the superior cervical ganglion.

With regard to the cells, it is misleading to speak of them as ganglion cells, even if they are nerve cells, since they have not the character of known ganglion cells. Their processes show no distinction of axon and dendrons, and no one has described other nerve fibres as making synapses with them.

It is hardly necessary to point out the improbability of the existence of nerve cells on the course of the sensory fibres of the iris. As to the cells supposed to be motor nerve cells, it may fairly be assumed that the figures given by Pollock represent the best results obtained. In his Fig. 8 the cells have the appearance of elongated nuclei attached to the nerve fibrils, and the cell given in Fig. 5 from an iris subsequent to the operation has processes stretching equally from opposite ends, about the length of those of a connective cell, instead of processes continuous on one side with a peripheral plexus.

The cells of the peripheral plexus of the iris are of the same type as those which are present in perivascular plexuses. The perivascular nerve fibres were found by Eugling(3) to disappear completely in a number of arteries in the denervated leg of the frog, and in the denervated ear of the rabbit. I obtained the same result(4) in the perivascular plexus of the main artery of the sartorius muscle of the frog, and observed the intermediate stage of the breaking up of the nerve filaments. Eugling found in the ear of the rabbit that the cells of the plexus remained as disconnected cells with short processes unconnected with the muscle fibres.

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Vitamines and lipoid metabolism. By W. CRAMER.

In a communication read before this Society three years ago(1) it was pointed out that there are two kinds of adipose tissue. Both these types contain in addition to the ordinary fat (unsaturated fatty acid esters of glycerine) deposits of double-refracting lipoids. The presence of lipoids can be recognised under the polarisation microscope and one of the groups of lipoids present can be identified as cholesterin by applying the Liebermann Burchard test to a chloroform extract of the dried tissue. These two types of adipose tissue are according to Hammar(2) histogenetically distinct and in the white rat and the tame mouse they remain microscopically and macroscopically distinct throughout adult life. In addition to the ordinary kind of adipose tissue there are found in these two species in certain definite sites sharply circumscribed masses of a brown fatty tissue. The main sites where this "brown fat" is found are: (1) at the back of the shoulders between the scapulae; (2) in the thorax along the aorta; (3) in the abdomen between the kidneys and the abdominal aorta and surrounding the adrenal glands which lie embedded in it. This tissue is very vascular which probably accounts for its brown colour. It is distinctly lobulated and gland-like in appearance. The fatty material instead of forming one large globule as in ordinary adipose tissue is arranged in a number of small globules around a central nucleus in a polygonal cell rich in protoplasm. This tissue differs histo-chemically and functionally from ordinary adipose tissue, histo-chemically in so far as it always contains lipoids with a varying amount of true fat, while in ordinary adipose tissue the true fat preponderates and there is a varying amount of lipoids. It differs functionally because the glandular lipoid tissue persists under conditions in which the ordinary adipose tissue is greatly diminished, such as a restricted diet, and conversely is not greatly increased in animals made obese.

When mice and rats are fed on a vitamine free diet of purified casein, starch, inorganic salts and olive oil, the fat disappears from the ordinary adipose tissue. According to Drummond(3) this is due to the absence of the water-soluble accessory factor in the diet. There is also a marked diminution of the contents of the glandular lipoid tissue, which, while persisting as a tissue may when the animal dies, be completely free from both fat and lipoid globules. Sometimes it is then deeply congested and of a purplish red colour to the naked eye.

In our previous paper attention was drawn to the fact that there is a close anatomical relationship of the perinephric glandular lipoid tissue and the adrenal cortex, and a great similarity between the nature of the lipoid contents of these two tissues. It is difficult to escape the suggestion of a functional relationship between these tissues, a suggestion which would raise the glandular lipoid tissue to the position of an endocrine gland. This view of a functional relationship finds further confirmation in the fact that in mice and rats dying as the result of being kept on the vitamine-free diet mentioned above, the lipoids of the adrenal cortex have almost completely disappeared and are restricted to the outermost layers of the zona glomerulosa. The medulla shows an almost normal load of adrenin. There is no sign of an active secretion.

In animals dying as the result of an absence of vitamines the temperature is sub-normal for several days before the animal appears seriously ill. This confirms an observation of Drummond's. According to him (4) this symptom appears only as the result of the lack of the water-soluble accessory factor and not when the fat-soluble factor alone is withheld.

A normal animal responds to a lowering of its temperature imposed upon it by an increased secretion of adrenin (5). Since the sub-normal temperature which appears in the course of a vitamine-free diet cannot be due to a lack of adrenin in the medulla, it must be ascribed to a paralysis of the functional activity of the active suprarenal gland. Here, as in other conditions where such a paralysis has been observed (gas gangrene), there is an almost complete disappearance of the lipoids from the cortex.

The observations reported in this note suggest that the "lipoid gland tissue," as it may be called, forms an important deposit of one or more of the vitamines and that disorders of the functional activity of the cells of this tissue may play an important part in the etiology of some of the deficiency diseases.

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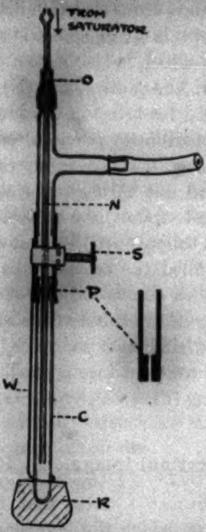
An apparatus for centrifuging blood without change of its gas content. By T. R. Parsons.

In modern investigations on blood there often arises the need for separating the corpuscles and plasma without disturbing the tensions of the gases of the fluid, so that the distribution of substances between corpuscles and plasma can be determined under definite conditions. The ordinary method of centrifuging below a layer of oil is open to objection on several grounds: firstly, carbon dioxide is by no means insoluble in most oils (cf. Barcroft, *Ergebnisse*, 7. 793), and secondly the oil also tends to produce a certain amount of hæmolysis; furthermore, if while the serum or plasma is being withdrawn a little of the oil enters the pipette, this usually sticks to the side and introduces a serious error into any measurement of the volume of the plasma which is to be made.

For these reasons the present apparatus has been devised in which blood can be centrifuged in a closed tube, which is either completely filled with the fluid or in which any remaining gas bubble is of such composition as to be in equilibrium with the blood. The centrifuge-tube itself is a simple glass tube (C) about 7·5 cms. long and capable of holding 1 c.c. At its open end it is provided with a short length of thick-walled capillary bore rubber-pressure tubing (P), and a specially small screw-clip (S). In making the latter the brass nuts and screws commonly sold for model-making were found to be very useful. This straight form of tube is found to be much less easily broken in the centrifuge than a more complicated pattern: the addition of a side tube, for example, introduces a point where unequal strains are set up and at which breakage is very liable to occur. The tube is protected while in the centrifuge by a thick pad of rubber (R), cut from a stopper. A stiff iron wire (W) is included in the clip and extends down until it ends in a loop against the rubber pad (R). In this way the whole system is kept rigid and the rubber tube (P) is prevented from being bent under the influence of the centrifugal force and so pulled or even torn off the end of the glass tube. The rubber tube (P) is only of capillary



bore for the length which is actually gripping the centrifuge tube; the remainder is hollowed out in the manner represented in the section alongside the main drawing. A rubber tube of this form is easily made from a piece of capillary bore pressure tubing by inserting a thin glass rod in it to serve as a guide, and then boring with a sharp cork-borer well wetted with glycerine for an appropriate distance. On withdrawing the cork-borer it is found that the glass rod is invariably pulled out inside it, and between the two is held the piece of loose rubber, so that the bore of the tube is enlarged for a certain distance to that of the cork-borer used. The object of this arrangement is to facilitate the operation of the filling attachment which is also shown in the figure, from which its action will be evident. The central tube (N) is connected at its upper end to the apparatus used for bringing the blood into equilibrium with the gas mixture, *e.g.* the saturator previously described (Parsons, This *Journal*, **51**. 443). During the saturation the gas passes down this central tube and up and out through the side piece of the T-tube. When the blood is made to follow, the central tube is slowly drawn up through the thick-walled tubing at (O), so that by the time the centrifuge tube (C) is completely filled the central tube is clear of the clip (S). The centrifuge tube is then clipped off and removed from the T-piece, and any blood remaining in the rubber tube above the level of the clip is removed. When the centrifuging is completed the plasma is withdrawn by means of a pipette drawn out into a capillary, which passes loosely through the rubber tubing (P).

The inversion of the retinal image. By H. Hartridge.

In a recent paper Senet(1) states that the retinal image is not inverted. The evidence on which the inversion of that image is based is absolutely reliable and may be briefly summarised as follows:

(1) If the eyeball of an albino animal be removed intact, and be mounted in a tube, so that while the rays from external objects enter the pupil, the posterior surface of the eyeball can be examined by an observer, then owing to the absence of pigment in the choroid the image formed on the retina is clearly visible. This image is seen to be inverted, top being at bottom and right being at left.

(2) In the case of an ordinary animal the choroid and sclera can with care be removed from the eyeball, leaving the retina *in situ*, observation of the retinal image shows that it is inverted and transposed.

(3) Histological examination shows that the exit of the optic nerve

lies to the nasal side of the fovea. The blind spot thus produced is found by experiment to lie to the temporal side of the point of fixation. It can be proved that the blind spot does correspond to the papilla of the nerve, by an independent observer *B* directing a beam of light with an ophthalmoscope into the eye of an observer *A*. It is found that if *B* directs the beam onto the blind spot that *A* only sees a faint diffuse glow, whereas if *B* directs the beam anywhere else *A* at once clearly sees it.

Further, if the angle subtended at the eye by the blind spot and the point of fixation be measured it is found to be equal to that of the fovea and the centre of the papilla of the optic nerve as measured from the posterior nodal point. This would seem to give conclusive evidence that the retinal image is transposed in man, and therefore since the optical system of the eye is a symmetrical one an inverted image also.

(4) Under certain pathological conditions opacities formed in the eye media cast shadows on the retina. These are seen projected onto external objects and may be located by the perimeter or Bjerum's curtain. It is thus found that opacities in one segment of the eyeball cause areas of blindness which occupy the opposite segment of the visual field.

(5) By applying a powerful light or sharply localized pressure to the exterior of the eyeball, as far from the summit of the cornea as possible a glow is seen projected onto the images of external objects. This is found to occupy the opposite segment of the visual field to that in which the stimulus has been applied.

The above summarises the evidence for the inversion of the retinal image and seems to me to be conclusive.

(1) Senet. Revista de la Universidad de Buenos Aires, 41. p. 398. 1919.

That the organ of Corti is dead beat. By H. Hartridge.

Perritt has advanced evidence from phonetics(1) that the organ of Corti is dead beat. The experiment described below gives additional evidence for that view.

A brass disc perforated by a circle of equidistant holes was mounted on a rotatable turn table. Against these perforations a stream of compressed air was directed by a nozzle, so that a syren was produced when the disc was rotated. One or more consecutive holes in the disc were now plugged with plasticene, and the disc rotated. The musical note produced was carefully listened to in order to determine if audible discontinuity in the note could be identified.

It was found that with such a speed of rotation that 400–600 puffs of air were produced per second, two consecutively obstructed holes caused a perfectly obvious period of silence, and that one obstructed hole was usually clearly audible. Calculation showed that the silent interval was on the average between one-one hundred and sixtieth and one-two hundred and fiftieth of a second. Such an interval should be so short that on Helmholtz's theory the resonators of the organ of Corti should bridge it without appreciable diminution in their amplitude of vibration. This interval is distinctly audible however and therefore some revision of the above theory would seem to be desirable.

(1) Perritt. Some Questions of Phonetic Theory. 1919. Chap. v. p. 33.

An operating table for class purposes. By H. Hartridge.

This table has been constructed so as to allow a room fitted up for the ordinary muscle nerve experiments, to be employed on isolated occasions for experiments on decerebrate and other animals. The design of the table therefore had to be such, that when not in use it would fold snugly up in a small space, and that it could be erected and dismantled easily and quickly.

It consists of a rectangular frame the centre of which is fitted with an electrically heated hot plate, one end of this frame bolts to the edge of the long tressel tables on which the recording drums, etc., for the ordinary muscle experiments are mounted, the other end being supported by a single leg, the lower end of which fits a hole in the floor, and which can be folded against the table when not in use.

The tracings were made on a long strip of smoked paper, one end being passed round a drum and the other round the drum next to it, the one being driven and the other turning idly.

Economical dehydrating and clearing agents. By H. Hartridge.

Amyl alcohol. For purposes of economy substitutes for absolute alcohol and clove oil were sought for. On Mr McCombie's suggestion amyl alcohol was tried. It was found that sections of moderate thickness could be passed from water to 95 % alcohol (for 1 minute) to amyl alcohol (for 1 minute), and then straight to the slide. The excess of amyl alcohol is then removed by draining or blotting and the section covered using Canada balsam in xylol in the usual way. In spite of this very rapid dehydration, no cloudiness, or other signs of the presence of water, developed.

Amyl alcohol has also been employed as a substitute for absolute ethyl alcohol for passing to No. 1 petrol (xylol substitute) for embedding in paraffin. It has been found satisfactory for both the above purposes.

No. 1 petrol as a substitute for xylol. Some pieces of tissue were passed from 95 % ethyl alcohol to amyl alcohol (for ½ hour) and to No. 1 petrol (for ½ hour) to melted paraffin (for 4 hours). After cutting on the microtome the sections were fixed to slides with egg albumen in the usual way and the paraffin then dissolved with petrol without the application of heat. No. 1 petrol can therefore be substituted for xylol in preparing sections of tissues.

PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY,

June 19, 1920.

The mode of action of vaso-dilator nerves. By G. V. ANREP and C. LOVATT EVANS.

Experiments were made on the tongue of the dog, in which vaso-dilatation was produced by stimulation of the lingual nerve: apart from the insignificant mass of lingual glands, and the extrinsic lingual muscles of the tongue supplied by the nerve (which latter have separate blood supplies), the only efferent fibres of importance supplied to this region by the lingual nerve are the hypothetical vaso-dilator fibres. This preparation therefore is a good test object on which the relation of vaso-dilatation to gaseous metabolism can be studied. Barcroft and Kato[1] have shown that in those cases where vaso-dilatation *is* accompanied by an increase in the gaseous metabolism, the effects of the stimulation only slowly die down.

The effects of stimulation of the lingual nerve on the blood flow through the tongue are more nearly confined to the period of application of the stimulus than is the case with a muscle on stimulation of its motor nerve as studied by Barcroft and Kato. The experiments were carried out on dogs: urethane and hirudin were administered, usually one or both hypoglossal nerves were cut, and cannulae were placed in the carotid artery and lingual vein for the collection of blood samples which were then analysed by Barcroft's method. Samples were collected during periods of rest, and during periods of stimulation of the lingual nerve by a faradic current of sufficient strength to give a pronounced increase in the rate of flow. In some cases where too strong a current was employed there was uncertainty as to the results because the oxygen usage was so small and the flow of blood so rapid that no difference could be detected between the arterial and venous bloods, either by the differential method, or by direct estimation of the total oxygen content of both bloods by means of ferricyanide. In most cases, however, small differences could be detected, and the results of four such experiments are given.

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[1] Barcroft and Kato. *Phil. Trans.* Sr. B. **206**. 149—182. 1915.

Exp.	Time	Condition	Flow of blood c.c./min.	c.c. oxygen used per min.	
1	5.2 p.m.	Rest	0·67	0·091	Mean of resting periods =0·119
	5.7 „	Stim.	5·0	0·095	
	5.35 „	Rest	1·0	0·175	
	5.45 „	Stim.	5·0	0·124	Mean of stim. periods =0·114
	5.55 „	Rest	1·02	0·122	
	6.00 „	Stim.	4·8	0·123	
	6.15 „	Rest	0·78	0·089	
2	4.12 „	Rest	0·83	0·152	
	4.20 „	Stim.	1·34	0·162[1]	
3	11.46 a.m.	Rest	0·253	0·054[2]	
	11.49 „	Stim.	1·88	0·073	Mean, rest =0·046
	12.17 p.m.	Rest	0·197	0·031	
	12.22 „	Stim.	1·03	0·046	Mean, stim. =0·058
	12.35 cut the other hypoglossal nerve				
	1.55 p.m.	Rest	0·34	0·054	
	2.2 „	Stim.	1·88	0·054	
4	3.40 „	Stim.	1·94	0·234	
	4.20 „	Rest	0·223	0·258	
Mean of all resting periods			0·59	0·144	
Mean of all stimulated periods			2·85	0·142	

[1] Hypoglossal nerves not divided, and slight movements of tongue occurred.

[2] One hypoglossal divided. Small fibrillar movements on stimulation.

Although metabolites no doubt play an important part in causing the vaso-dilatation associated with functional activity, these experiments indicate that there is a likelihood that vaso-dilator nerve fibres actually exist. These could possibly be thrown into action reflexly, as in vaso-dilator reflexes. But the experiments do not throw any light on the question whether powerful vaso-dilator metabolites may be formed by non-oxidative cleavage processes in the tissues.

Influence of increased water ingestion on blood-pressure. By J. B. Orr.

In recent years there has been an accumulation of evidence that the presence in the blood stream of certain disintegration products of protein that arise from bacterial action cause a rise in blood-pressure. (Dixon and Taylor(1), Barger and Dale(2) and others). It has been shown by Dale and Dixon(3) that these pressor substances may be absorbed from the alimentary canal. It appears that they may also arise by other means than through bacterial action. Emerson(4) obtained a pressor substance in the autolysis of pancreas under conditions that excluded putrefaction. These results indicate that certain bodies that cause a rise in blood-pressure may arise in the intestine from bacterial action on protein and be absorbed to the blood stream. They suggest also the possibility that pressor substances may originate in the tissues through incomplete or perverted metabolism.

Fowler and Hawk(5) showed that increased water ingestion produced a decrease of bacteria in the fæces. It has been shown (J. B. Orr(6)) that the increased passage of water through the system promotes an acceleration of the catabolic and synthetic phases of protein metabolism. If these results be correct increased ingestion of water should tend to eliminate pressor bodies whether arising in intestinal bacterial activity or in defective protein metabolism in the tissues. A series of experiments which has been carried out appears to show that a fall in blood-pressure does follow an increased water consumption.

In these experiments readings were taken during both "pre-water" and "post-water" control periods when the subject neither curtailed nor increased his water consumption. On the "water" day 3 litres extra were drunk. The readings were taken with the subject lying on a bed and after 15 minutes rest. Corresponding readings were taken at the same hour of the day and meal times were fixed. The instrument used was a Riva-Rocci with a screw compressor which allows a fine adjustment. The auditory method whose accuracy has been conclusively demonstrated by MacWilliam, Melvin and Murray(7) was adopted.

		Noon Readings			Afternoon Readings		
		Syst.	Diast.	Pulse	Syst.	Diast.	Pulse
Subject J. B. O.	Pre-water	112	58	76	112	60	72
age 38	Water	109	59	80	114	63	62
6th Exper.	Post-water	106	58	80	111	48	76
Subject J. R. H.	Pre-water	112	68	60	104	62	60
age 31	Water	111	70	50	101	61	66
3rd Exper.	Post-water	106	61	50	97	56	48
Subject J. I. M. I.	Pre-water	126	76	58	127	77	62
age 21	Water	134	84	64	118	64	60
1st Exper.	Post-water	123	69	54	126	69	66
Subject J. I. M. I.	Pre-water	125	60	60	119	68	58
age 21	Water	115	74	60	126	64	72
3rd Exper.	Post-water	117	65	60	116	64	60
Subject J. L.	Pre-water	148	86	60	134	83	56
age 30	Water	137	88	60	136	88	54
1st Exper.	Post-water	142	87	54	134	87	50
Subject J. L.	Pre-water	133	78	64	127	79	72
age 30	Water	133	79	61	129	75	60
3rd Exper.	Post-water	126	75	60	123	73	66

The results seem to show that a fall which is variable in extent tends to occur in both systolic and diastolic pressures after the water has passed through the system. In two subjects who had a relatively high pressure, there was an increase on the day on which the extra water was drunk. This was most marked in the first experiment. In one of these subjects the "post-water" fall was not definitely obtained until the third experiment.

To economise space in this preliminary communication the readings are only given for (1) the day before the extra water was taken, (2) the water day, and (3) the first day following. In No. 1 subject, J. R. H., the results of the first water day only are given though extra water was taken in three days. In the others extra water was taken only on one day. In the case of the two subjects with an initial high pressure the results of the first and third experiments are given. Readings are in mm. mercury.

REFERENCES.

(1) **Dixon and Taylor.** B.M.J. 2. 1150. 1907.
(2) **Barger and Dale.** Jl. Physiol. 41. 19. 1910.
(3) **Dale and Dixon.** Jl. Physiol. 39. 25. 1909.
(4) **Emerson.** Beitr. Chem. Physiol. Path. 1. 501. 1902.
(5) **Fowler and Hawk.** Jl. Exper. Med. 12. 388. 1910.
(6) **Orr.** Biochem. Jl. 8. 530. 1914.
(7) **MacWilliam, Melvin and Murray.** Proc. Phys. Soc. March 1914.

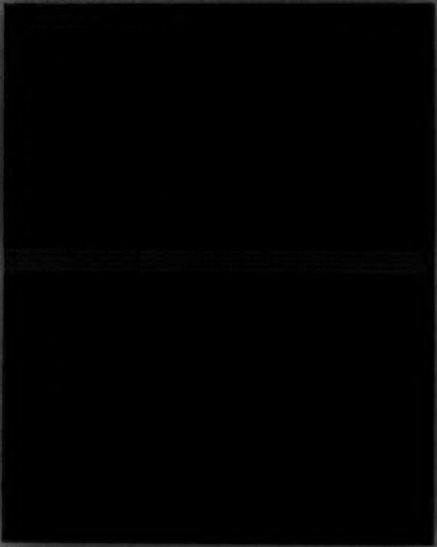
The use of the thermionic valve with the string galvanometer.

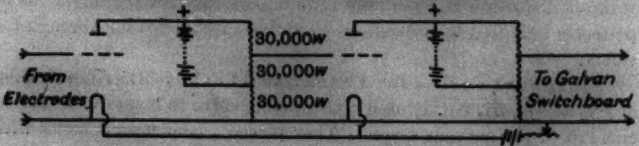
By I. de Burgh Daly (*Beit Memorial Research Fellow*) and K. E. Shellshear. (*Preliminary Communication.*)

In view of the fact that some physiological currents are too small to be recorded by the string galvanometer it was thought that their magnification would be of interest. The Thermionic Valve, which has been almost universally used during the war for amplification of wireless signals, seemed to hold out possibilities in magnifying these physiological currents. An aperiodic circuit which would amplify electrical variations without distortion is essential and it is necessary for the circuit to be perfectly balanced to prevent an excessive current flowing through the string.

Alexander Forbes and **Catharine Thacker** (*American Journal of Physiology*, **51.** No. 1) used a thermionic valve to magnify the action currents of the nervous system. They placed a condenser in series with the string to protect it from the large current flowing from the plate batteries and this would have the effect of flattening out the curves.

The valve placed in one arm of a Wheatstone bridge as described by Dr W. H. Eccles (*Nature*, **104.** No. 2620), was the method eventually used and we are much indebted to Dr Eccles for valuable help. As a criterion of the aperiodicity of the circuit we used the electrocardiogram although it was recognised that magnification of the electrocardiogram *ipse se* would not lead to any useful information. Two curves are given showing the difference in amplitude of the string excursion of the normal electrocardiogram and of the electrocardiogram magnified by using two





valves. The tension of the string remained the same in both curves. The plate voltage was 66, filament current ·8 amp., and the three resistances 30,000 ohms. The valves used were the Marconi V. 24.

The ripples on the magnified electrocardiogram are being investigated and it is hoped that they may be overcome. We wish to express our thanks to Professor Bayliss for many helpful suggestions.

The action of potassium and uranium on the frog's heart. By A. J. CLARK.

Zwaardemaker(1) has shown that if a frog's heart is arrested by perfusion with potassium free Ringer, it will recommence a regular beat on the addition of uranium, or any other radio-active salt, to the perfusion fluid. He concludes that "the potassium atom may, as regards function, be replaced by all other radio-active elements."

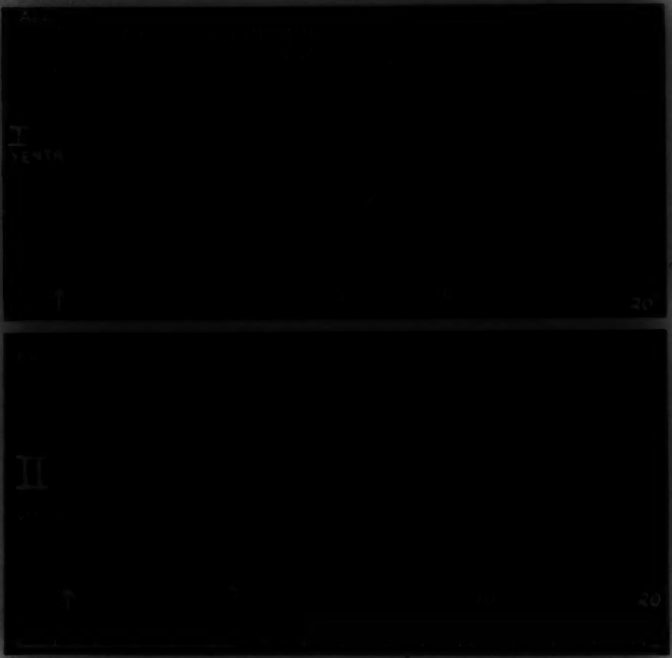
I consider that this conclusion can only be accepted with considerable qualification.

I have tested the action of uranium upon the excised frog's heart; the hearts were perfused with one cannula in the sinus and one in the aorta, the Ringer used contained NaCl 0·65 p.c.; $CaCl_2$ 0·01 p.c.; KCl 0·01 p.c.; $NaHCO_3$ 0·02 p.c.; $P_H - 7{\cdot}7$.

When the potassium in this fluid was replaced by rubidium chloride (0·015 p.c.) no certain alteration could be observed in the activity of the heart. When the potassium was replaced by 0·02 p.c. cæsium chloride the heart showed the usual signs of potassium lack, and stopped in about half an hour. The alterations on its activities were not so rapid as when simple potassium free Ringer was perfused, and therefore cæsium can compensate for potassium lack to a slight extent. These facts were observed by Ringer(2).

When the heart is perfused with potassium free Ringer a very well defined series of changes in its activities occurs; firstly, the length of the mechanical response of the auricle and of the ventricle increases, and at the same time the length of the a.–v. interval increases; after a few minutes a partial auriculo-ventricular block occurs, and is followed by a sinoauricular block; at the same time the tonus increases, and diastole is imperfect; finally, when the auriculo-ventricular block becomes complete, the ventricle is arrested in diastole, and the auricular beats cease shortly afterwards, the arrest being complete in about 30 minutes. The sinus continues to beat for at least an hour afterwards and the ventricle will respond to direct stimulation for more than an hour afterwards.

When a heart is perfused with Ringer in which the potassium has been replaced by uranyl nitrate (0·0001 – 0·0025 p.c.), all of the above changes occur, *i.e.* the heart first shows systolic effects and then becomes arrested in diastole, the arrest persisting for the duration of the experiment, which in some cases was for 3 hours; in fact I was unable to detect any certain difference between the effect of perfusing with



Action of lack of potassium, and of lack of potassium in presence of uranium upon the isolated frog's heart.

Tracing I shows the action of lack of potassium. Potassium free Ringer was perfused at the arrow.

Tracing II shows the effect of uranium. At the arrow a Ringer's fluid was perfused containing no potassium but 0·002 % uranyl nitrate.

The effect in the two cases is identical, both hearts stopped in diastolic arrest in 20′, and remained arrested permanently.

In both tracings the upper curve records the auricular and the lower curve the ventricular contractions. Up-stroke = systole. Time in 10 seconds.

potassium free fluid and with potassium free fluid plus uranium. This result is not due to uranium having a long latent period before it acts, for if the heart is first perfused with normal Ringer plus uranium for an hour, and then with potassium free Ringer plus uranium, exactly the same changes as described above occur when the potassium is removed. The addition of uranyl nitrate to normal Ringer in quantities up to 0·01 p.c. produces no effect upon the heart in an hour.

Some of these changes are shown in the figure.

When a heart is perfused with KCl free Ringer until complete arrest is obtained, then the addition of uranium will frequently cause it to resume an irregular beat, but I have never been able to obtain a beat of normal frequency, and with a normal a.-v. interval, and a normal length of mechanical response. Zwaardemaker states that under these conditions he has obtained a beat of normal frequency and normal force of contraction, but the tracings published in his papers are not sufficient to judge his results.

I performed similar experiments with the isolated auricle of the rabbit, in this case lack of potassium causes an initial increase in frequency and force of contraction, and finally arrest; when Ringer minus potassium, plus uranium 0·005 %, was perfused, exactly the same changes were observed as with potassium free Ringer.

I consider therefore that, although uranium will excite a heart arrested from lack of potassium, yet it cannot be said to replace potassium in the manner in which rubidium will replace potassium.

(1) **H. Zwaardemaker.** Journ. Physiol. 52. p. 273. 1920.
(2) **S. Ringer.** Journ. Physiol. 4. 370. 1884.

Graphic conversion of Sörensen's p_H (− log [H·]) **into concentrations of hydrogen ions.** By H. E. Roaf.

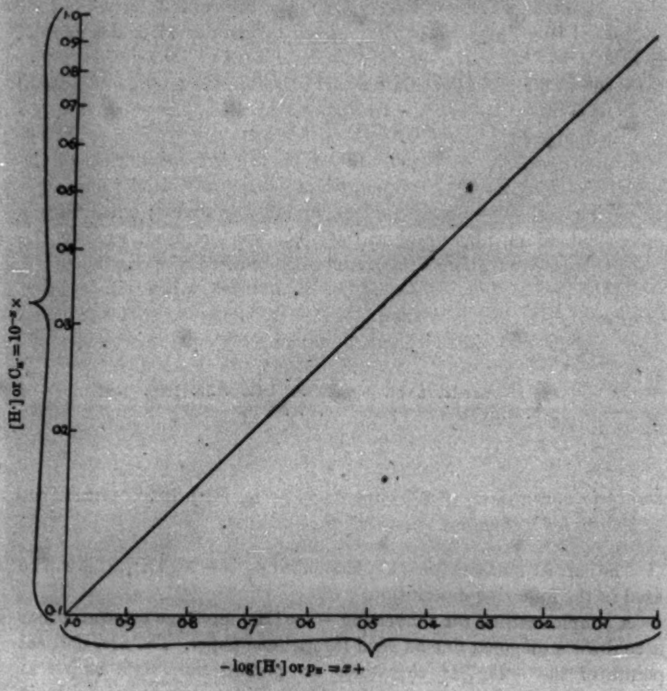
The correct mathematical expression for p_H is − log [H·]: this will be used in the following description.

A simple method for converting − log [H·] into true concentrations is to draw a diagonal line on semi-logarithmic paper. The first decimal points of the − log [H·] expression are plotted from right to left as abscissæ and the concentrations from 0·1 to 1·0 against the logarithmic rulings as ordinates. The whole number of the − log [H·] gives the negative power of ten by which the concentration must be multiplied.

In the absence of semi-logarithmic paper squared paper can be used only the concentrations must be marked against the lines corresponding to their logarithms: the semi-logarithmic paper is more convenient as one can read from it correctly to two places of decimals.

For example to convert $-\log[H^{\cdot}] = 6{\cdot}7$ into concentrations of hydrogen ions, 0·7 cuts the diagonal line opposite 0·2, therefore the concentration of hydrogen ions is $0{\cdot}2 \times 10^{-6}$.

One can avoid the use of $-\log[H^{\cdot}]$ by plotting the electrical potentials against hydrogen ion concentrations on semi-logarithmic paper.



On the so-called growth of amputated parts of plants. (*With Demonstration.*) By A. D. WALLER.

A report has been published by Professor Bayliss and seven other distinguished authorities of a demonstration made to them by Sir J. C. Bose's "crescograph" on Friday afternoon, April 23rd, in the Physiological Laboratory of University College, London. That report as indicated in the current issue of *Nature* (May 6, p. 305) is to the effect that the instrument correctly records changes of length in the growing tissue, or indeed of any substance attached to the lever of the instrument however such changes may be produced. It is however not clear from the terms of the report whether the correctness of the instrument is regarded as comprehensive of all and any kinds of elongation collectively, or to the elongation due to true growth isolated from elongation due to other causes.

Moreover the University College demonstration is reported as having been made upon an "actively growing bud," whereas the demonstrations previously made at the Royal Society of Medicine and elsewhere were performed upon the apical parts of leaves (daffodil, hyacinth and cyclamen).

The statements of the University College Committee have been quoted by Sir J. C. Bose in contradiction of a previous statement made by me dealing with what I actually saw and recognised as being a perfectly familiar fallacy (*Transactions of the Royal Society of Medicine*, 1920). The demonstration made by Sir J. C. Bose at the Royal Society of Medicine on March 11th, was of an entirely different character. So-called growth of normal speed was "demonstrated" upon parts of plants devoid of any growth zone in which therefore such growth was impossible. An amputated leaf of a hyacinth (or daffodil) was shown and stated to exhibit "growth"; it was then "stimulated" by induction shocks and stated to exhibit arrest and reversal of growth. My opinion that the demonstration was illusory is shared by Mr Harold Wager, who was also present and who writes to me as follows: "I am quite at a loss to understand upon what grounds he (Sir J. C. Bose) regards the movements shown by his apparatus as being movements of growth. Variations of turgidity in vegetable cells which may be very considerable are not necessarily indicative of growth any more than the movements of a pulvinus under stimulus are growth movements. Moreover I noticed that Bose took apical pieces of the leaves (either daffodil or hyacinth—I forget which) in order to demonstrate the growth with his apparatus.

Now it is a well-known fact that the growth of such leaves is basal not apical and experiments which I have made—rough certainly—as compared with those of J. C. Bose—do not show any growth at all in the apical region, or indeed in any part of the leaf except just at the base."

The elongation of a bit of plant as shown at a magnification of 1000 (and *a fortiori*, of 1 to 10 millions) may be due to growth or to some physical fallacy such as change of temperature, slipping of attachment, increase of turgor, etc. The most fruitful source of fallacy is turgor, any suitable bit of plant rapidly loses turgor and becomes flaccid and if its cut end is placed in water or clamped in wet cotton wool it gradually recovers turgor and exhibits elongation, whether it grows or not. The amputated leaves of hyacinth, daffodil, lily, iris, etc. grow only at the base and elongate only by increase of turgor (or other physical fallacy). The amputated buds of lily, hemlock, lupin, etc. contain growth zones, and can elongate partly by true growth, partly by increasing turgor (or other physical fallacy).

The first point that requires investigation before the study of growth proper can be profitably undertaken, consists in the experimental distinction between the elongation due to increased turgor and that attributable to true growth. Broadly speaking the distinction is simple, turgor is a reversible change, gain or loss of water causes gain or loss of length. Growth is an irreversible change and is attended by continuous gain of length not followed by loss of length ("degrowth"). If two rapidly growing buds (*e.g.* the flower spikes of Lupin) are set up side by side and arranged to record simultaneously their elongation for a minute or two, it will be seen that the bud in which turgor is maintained (stalk in water) grows rapidly (0·25 μ per sec.), whereas the bud that loses turgor (stalk in air), grows more and more slowly, then stops, and finally shortens. The effects of electrical excitation will be dealt with in a further communication.

Growth true or false is most easily demonstrated and recorded at a magnification of 1000, when actual elongations of 10, 20, ... are represented to the eye by elongations of 10, 20 ... mm. Thus, *e.g.* the apical half of an iris leaf showed no elongation whatever during 24 hours, whereas its basal portion showed an apparent elongation of 60 mm. during five minutes, *i.e.* exhibited growth at a speed of 0·2 μ per second.

PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY,

July 10, 1920.

A peculiar effect of chloroform on the cardio-inhibitory centre.

By J. B. Collip.

On an occasion during a mammalian demonstration in which a dog was used, the anæsthetic was abruptly changed from ether to chloroform. It was noted that shortly after chloroform anæsthesia had been initiated a peculiar type of vagus pulse became manifest in the tracing of the mercury manometer which was connected with the left carotid artery (Fig. 1). This effect at once disappeared when ether anæsthesia was resumed and reappeared when the ether was again substituted by chloroform. As it was quite evident that the phenomenon was due to the inhalation of chloroform, the vagi were cut to determine whether the

Fig. 1. Blood-pressure tracing from a dog under ether: at the signal chloroform was substituted for ether. Time tracing in 10 sec. intervals.

effect was of cardiac or vaso-motor-origin. The section of both vagi abolished the effect (Fig. 2). The respiration, the record of which was taken but is not here reproduced, showed nothing to account for this variation in blood-pressure. It would therefore appear that there was in this animal a rhythmical alteration in the tonus of the vagus centre in the medulla caused by the direct action of the chloroform.

As yet I have been unable to duplicate these results.

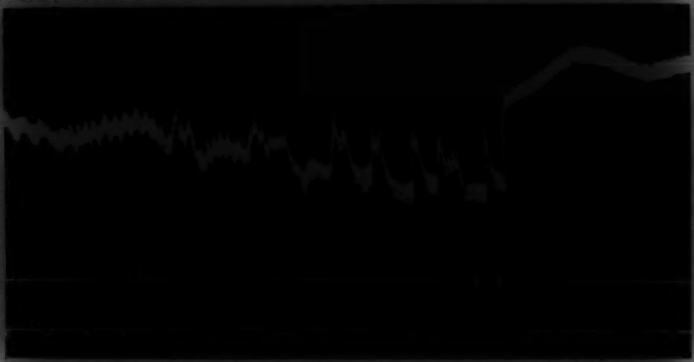


Fig. 2. Blood-pressure tracing from dog under morphia and ether: at the first signal chloroform was substituted for ether: at the second and third signals the two vagi were cut. Time tracing in 10 sec. intervals.

Respiratory surface in crabs. By G. C. C. Damant.

The following measurements of the total gill surface in crabs are put on record because these animals (*a*) have gills which lend themselves to accurate measurement, (*b*) there is no reason to suppose the surface to have any function (capture of food, etc.) other than the respiratory one, and (*c*) the integument of the animal is such that it cannot take any appreciable part in gaseous exchange. Hence the gill surface of this animal may be fairly considered to represent the total respiratory surface required by an active marine invertebrate.

Method.

The gills consist of pyramidical piles of heart-shaped lamellæ resembling the leaves of a book, which (in *Carcinus maenas*, the common shore crab, at any rate) are comparatively rigid structures standing manipulation, alcohol, etc. without change of form.

The gills were removed and the number of lamellæ in each counted under a dissecting lens. A lamella was then removed from that part of each gill which represented the average area and mounted so that its enlarged image could be projected on to squared paper and the area measured. This area (doubled to allow for both upper and lower surfaces) multiplied by the number of leaves in that gill gave the total surface of the gill. Each of the nine gills of one or both sides of the crab were measured in the same way and it was found that in *Carcinus maenas* (the common shore crab) the average gill surface is 7·7 square centimetres per gramme of body weight.

A similar ratio exists in the case of *Cancer pagurus* (the red edible crab) and *Portunus* sp. (the swimmer crab) of which single specimens were examined, giving a surface of 7·8 and 8·7 sq. cm. respectively per gramme of body weight.

TABLE I. *Showing the total and relative gill surface in five specimens of Carcinus maenas.*

Sex and weight	Total gill surface	Gill surface per one gramme of body weight
♀ 23 grammes	190 sq. cm.	8·3 sq. cm.
♂ 33 „	287 „	8·7 „
♀ 48 „	462 „	9·6 „
♂ 75 „	535 „	7·1 „
♂ 102 „	675 „	6·6 „

TABLE II. *Detailed measurements of surface of the gills of one side of a ♂ Carcinus maenas weighing 75 grammes.*

Gill	Number of lamellæ	Surface of average lamella	Total surface of that gill
1st	95	0·103 sq. cm.	9·8 sq. cm.
2nd	114	0·050 „	5·7 „
3rd	44	0·103 „	4·5 „
4th	134	0·155 „	20·8 „
5th	115	0·308 „	35·4 „
6th	152	0·288 „	43·5 „
7th	138	0·407 „	56·3 „
8th	133	0·364 „	48·4 „
9th	129	0·302 „	39·0 „

Adding the surfaces together gives 263·4 sq. cm. as the total gill surface of one side and, since the gills are quite symmetrical bilaterally, 526·8 sq. cm. would be the total respiratory surface of the crab; this works out at nearly 7·0 sq. cm. per gramme of body weight.

The capillary blood-pressure. By LEONARD HILL.

Observations have been carried out on the measurement of capillary blood-pressure in cold-blooded animals:

1. In the tadpole's tail.
2. In the toad's lung, tongue and mesentery.
3. In the frog's lung, tongue, mesentery and web.
4. In the bladder of the frog and toad.
5. In the newt's lung and mesentery.

The apparatus used is that of Roy and Graham Brown (*Journal of Physiology*, **2**, p. 323. 1879–80).

A small cylindrical brass box is constructed[1], with a side tube and a glass floor. On to the top of the box is tied a piece of transparent peritoneal membrane. The membrane is not stretched tight, but tied on so that it has a dome-shaped form. The brass box is surrounded with cork, so that the part which is under examination can be pinned out, *e.g.* the web of the foot, so that the transparent web lies over the top of the brass box in contact with the wet peritoneal membrane. By means of a sliding holder a piece of glass is brought down so as gently to touch the wet upper surface of the web. The side tube is connected by a T-piece with a water manometer, and with a length of rubber tubing closed at the distal end. The tubing can be so compressed as to raise the pressure in the brass box, and compress the web of the foot between the peritoneal membrane and the piece of glass. The manometer indicates the pressure which is applied. In estimating the capillary pressure it is not just to take that pressure which shuts up the capillary and prevents flow, but the lowest pressure which, quickly applied, distinctly modifies the rate of flow.

If a capillary net-work is shut up the pressure within it must rise to that within the arteriole which is supplying it.

Take the case of the tadpole's tail, when the distal part is compressed the blood-flow will be stopped at the pressure which causes the blood to take the pathway through the arterioles supplying the net-works in the proximal part which is uncompressed.

Again in the case of the lung or bladder, when the whole of the distal part is compressed, one cannot stop the flow until the pressure is greater than that in the arterioles supplying this part. As the pressure is raised—in the case of the lung for example—one sees the flow become pulsatile alike in arterioles, capillaries and veins; the whole mass throbbing, and finally the pulse failing to pass through these vessels at one and the same time when the pressure over-tops the systolic pressure. The whole of the phenomena which happen in the application of the armlet of the sphygmomanometer to the human arm, in measuring blood-pressure, can be observed then in a frog's lung when compressed, and in a most instructive manner. The pressure which it takes to stop the systolic pulse passing through is, as Roy found, some 25–30 cm. of water; the "diastolic" pressure may be determined at the point when the pulsation ceases to be marked as the pressure is lowered, *e.g.* in one case of a toad's lung, for example the readings were:

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[1] I am indebted to Dr E. Schuster for making this apparatus.

Systolic 31 cm. water.
Diastolic 23 „ „

The pressure required to modify the capillary flow in the tadpole's tail, *i.e.* the capillary pressure, may be as low as ·5 cm. of water in outlying capillaries. 1 to 3 cm. of water are pressures which generally modify capillary flow in the animals examined.

There are some capillaries which require higher pressure than this, *e.g.* 5–6 cm. of water, such as the capillaries in the papillæ of the frog's tongue. The substance of the tongue probably resists compression. On finding a net-work in the tadpole's tail or frog's web supplied by an arteriole in which the flow is modified by 1 cm. of pressure, in an adjoining net-work, supplied by another arteriole, and with a more rapid flow, perhaps 3 or 4 cm. may be required to modify the flow: but on the whole, as the result of the examination of a large number of animals, the conclusion reached is that the capillary pressure in cold-blooded animals[1] is exceedingly low, and never approaches anything like the figure which is usually given in the text-books. The flow in the veins is momentarily slowed by a pressure which is as low as that found in the capillaries. In the mesentery, etc. the arrest of flow in vessels under compression, the reversal of flow by compression, and the setting up of flow through anastomatic pathways can be studied. Roy's method is a most valuable one then for instructing students in the principles of the circulation.

If the leg of a frog after ligation is excised and the web placed on the apparatus an intermittent pressure of 2–3 cm. of water will cause a to and fro flow no less rapid than the normal flow. In such a web local flow may occur through slight variations of pressure due to posture, etc.; application of warmth may start such a flow.

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[1] The same holds good for the capillaries in the ear of a mouse.

A rotating contact breaker designed by Keith Lucas. By E. D. ADRIAN.

For the investigation of conducting tissues which approach the reflex arc in complexity it is generally necessary to employ a series of stimuli, and in order to control all the variable factors in stimulation we must be able to alter independently the form (*i.e.* the strength and duration) of the individual stimuli and also their frequency. In 1914 Keith Lucas constructed a rotating contact breaker which interrupts a constant current and produces a series of stimuli of which the duration and the frequency can be rapidly and independently adjusted over a wide range. The general arrangement of the instrument is shown in Fig. 1.

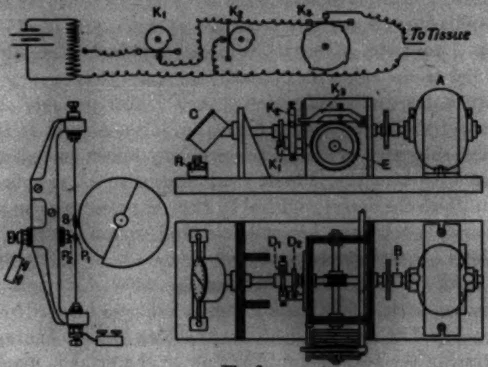

Fig. 1.

A motor *A* rotates the main shaft *B* at a constant speed controlled by a rheostat *R* and measured by the speedometer *C*. On the main shaft are two cam wheels D_1 and D_2 which open and close two contact keys K_1 and K_2 once in every revolution. The keys are specially designed for working at high speeds: a light steel wire is tightly stretched on a brass frame by a set screw, the wire carries a small steel Shoe *S* which engages with the surface of the cam, and a platinum contact P_1 which is forced up against the platinum point P_2 when the cam presses the shoe outwards. When the step in the cam is reached the shoe *S* falls back and contact is broken. As the inertia of the wire is very small and its tension high, the shoe will follow the contour of the cam faithfully without lag or overthrow although the cam wheel is running at speeds as high as

60 revs. a second. The stimulating current is derived from a potentiometer and the two keys are connected so that K_1 is an in-circuit and K_2 a short-circuit key; thus the current starts flowing through the main circuit when K_2 is opened and stops when K_1 is opened, and the duration of each stimulus can be varied by altering the angle between the two keys.

The frequency of stimulation is varied by a second series of cams *E* driven by a worm reduction gear at $\frac{1}{60}$ of the speed of the main shaft. There are ten cam wheels with 30, 20, 15, 12, 10, 6, 5, 4, 3, and 2 cams on their circumference, and a single contact key K_3 on a sliding rod can be brought into contact with any of the wheels. The cams are arranged so that K_3 is closed during every 2nd, 3rd, 4th, 5th, 6th, 10th, 12th, 15th, 20th or 30th revolution of the main shaft, and as K_3 is in the main circuit the currents generated by K_1 and K_2 can only reach the tissue when K_3 is closed. Thus the frequency of stimulation can be made $\frac{1}{2}$, $\frac{1}{3}$, $\frac{1}{4}$, etc., of the frequency of revolution of the main shaft by shifting K_3 from one cam wheel to another. Both the frequency and the duration of each stimulus can be altered in a few seconds whilst the motor is running, and the exact values can be determined at once by reading the speed of the motor, the angle between K_1 and K_2 and the position of K_3.

The form of the current waves is shown in Fig. 2 which gives a record of the potential difference at the electrodes measured by analysing a capillary electrometer photograph. Each stimulus lasts ·0025 sec. and the interval between them is ·0155 sec. With the same speed of the motor it would be possible to vary the duration of each stimulus from ·00015 sec. to ·0039 sec. and to increase the interval between them to ·465 sec. Other ranges could be obtained by running the motor at a slower speed. Incidentally the record shows the accuracy of the capillary electrometer in measuring very rapid changes of potentials.

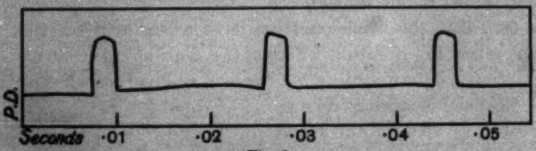

Fig. 2.

The excretion of phosphoric acid in the urine. By H. C. BROADHURST, Beit Memorial Research Fellow, and J. B. LEATHES.

Figures have been obtained in earlier experiments which show that there is a remarkable variation in the rate at which phosphoric acid is excreted in the urine at different times in the day.

Cathcart, Kennaway and Leathes[1] published figures from which it may be seen that if the maximum rate, which occurred always in the evening, be put as 100 the minimum rate, which occurred always in the forenoon, will figure as 48, 39 and 32 in the three subjects respectively. These results are the average from a number of consecutive days, 16, 26 and 27 respectively, during which the diet was uniform and the same.

In a long series of water tests carried out on patients suffering or recovering from war nephritis and on normal persons the output of phosphoric acid was determined in about a hundred cases before and during the diuretic response. Details will appear elsewhere: it is sufficient here to note that whatever the degree of diuresis the hourly output of phosphoric acid was invariably less during the diuresis (in the forenoon) than it had been during the night before the water was taken. This holds for normal subjects, in whom the hourly output of water increased six fold, in patients recovering from nephritis in whom it increased to from four to eight fold and in those who showed little or no diuretic response. In the different groups of cases the diminution of the hourly output of phosphoric acid varied from 25 to 50 p.c. the latter figure being the one obtained in normal subjects.

The fact that the urine tends to have a less acid reaction in the forenoon, raises the question whether the diurnal curve of phosphoric acid excretion is determined by the curve of acidity[2], or whether it has a physiological significance of its own.

Moreover in view of the results of Trevan[3] who found that the perfusion of the frog's kidney with Ringer's solution gave an alkaline urine while perfusion with a phosphate solution of a certain slightly alkaline reaction gave a secretion that was more acid, a different question arises, whether the greater acidity of the urine in the afternoon and night may not be due to the fact that there is more phosphate to be excreted at this time of day. In other words, is the diurnal tide in the reaction of the urine produced by the phosphate tide, or is the variation in the rate of excretion of phosphoric acid a result of the alkaline tide, or are the two phenomena distinct and independent of each other?

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[1] *Quarterly Journal of Medicine*, 1. p. 416. 1908.

[2] Cf. Magnus Levy, in von Noorden's *Pathologie des Stoffwechsels*, Bd. I. p. 43.

[3] Trevan. This *Journal*, 50. p. xv. 1916.

Some experiments have now been carried out to see whether the output of phosphoric acid can be diminished by procedures which are known to affect the reaction of the urine. Forced breathing performed for a few minutes will make the urine alkaline at a time when the secretion is normally acid. If this is done at a time when the phosphate output normally rises, about noon, the rise may be diminished or delayed but is not effaced even by a considerable change in the reaction of the urine in the direction of alkalinity. If this is done at a time when the phosphate output is high, in the evening or at night, the hourly output is diminished but will remain higher than at the slack of the phosphate tide although the reaction of the urine is made far more alkaline by the hyperpnœa than it is at that time otherwise.

The time of day at which the phosphate output is highest suggests further that it is so because the intake of phosphorus with the food taken during the day causes larger amounts to appear in the urine as the day advances.

But that this does not explain the phenomenon, though it contributes an element in it, is shown by the rates observed in three normal subjects who took no food for a period of thirty-six hours: in all three cases the output of phosphate was lowest in the early hours of the day and rose very considerably in the afternoon to values double those observed earlier, or even more than that. The stoppage of intake of phosphorus may cause the minimum rate to occur earlier in the morning but leaves the maximum rate in the afternoon a conspicuous feature of the curve.

The ebb and flow of phosphate output is therefore an independent phenomenon although the reaction of the urine is not without effect upon it. Whether it is due to more brisk metabolism of nerve or of muscle or other tissues during the waking hours these experiments do not suffice to show. Other experiments are necessary also and are in progress for the elucidation of the relation between phosphate output and the reaction of the urine.

A note on the brown granules found in some endocrine organs.

By R. K. S. Lim.

These granules have been noticed in the suprarenal cortex of the cat, the corpus luteum of the rabbit and the pars nervosa of the human hypophysis. In unstained formol-fixed preparations they appear brown, but with methylene blue they take on a deep green colour. Other dyes have no characteristic effect. They are not lipoids nor do they contain iron, and they occur in the connective tissue cells of the organ in which they are found.

In the cat suprarenal, these granules occur in the connective tissue under the capsule and in the trabeculæ which pass down towards the medulla; they may even be found in isolated masses within the zona reticularis. They have been observed in all the six cats examined, and in most amount in a pregnant animal. None, however, could be seen in the suprarenals of the dog, rabbit and rat.

Similar granules were found in the cicatrized centre of the corpus luteum of two rabbits. The granules gave the typical reaction with methylene blue and were contained within irregularly branched cells.

The two human pituitaries examined were from recent cases of hæmorrhage in the brain stem. The granules were present in a great many of the cells of the pars nervosa. Their appearance resembled that seen in the two former regions. Pituitaries of the cat and ox do not contain them.

Examination of the other organs and of the areolar tissue from various regions in the same animals have been negative.

Nutrition on diets practically devoid of fat. By J. C. DRUMMOND.

Much discussion has been devoted to the question of the dispensability of fat in the diet of the mammalian organism, but no definite decision has been reached in the absence of experimental proof. Earlier researches have, however, yielded inconclusive results owing to the effect of complications which were not then appreciated. Stepp's experiments showed that an addition of neutral fat would not restore the nutritive value of foods which had been extracted with alcohol and ether, and, further, Osborne and Mendel reported that young rats would live and show growth for some time when fed upon artificial food mixtures which were practically fat-free. These results led up to the discovery of the fat-soluble accessory food factor; a discovery which made it obvious that the solution of the problem of the dispensability of fat could not be achieved until it was possible to prepare a diet containing the factor A, but no neutral fat. During the research which is briefly reported here many attempts were made to prepare a fraction from butter or cod liver oil, which should satisfy these requirements, but without success. Dr Zilva, of the Lister Institute, was, however, kind enough to inform me that he had prepared a highly active fraction by extracting fresh carrots with alcohol. I have prepared a similar extract, which contains only the smallest traces of neutral fat, but which is fairly rich as a source of the factor A.

Young rats weighing about 50 gms (4 wks old) were fed on a basal ration composed of caseinogen and starch, both rendered as fat-free as possible by repeated extraction with alcohol and ether, inorganic salts, orange juice and yeast extract. Of these constituents only the yeast extract contained a small amount of substance soluble in ether. In addition to this diet, the animals received a daily ration of 5 c.c. of the concentrated carrot extract, which was administered before their day's food was given.

All the animals except one remained in good health for nearly six months, and showed considerable growth, although the rate was irregular and on the whole sub-normal. Unfortunately these experiments were interrupted before a conclusive result could be obtained, but it would appear probable that the mammalian organism can exist without receiving fat in the food. At the close of the experiment the animals were in good health, and showed no signs of the decline which is associated with an absence of the fat-soluble factor A. Considerable deposits of body-fat were found at post-mortem examination.

The diet was not strictly free from neutral fat, as the following figures will show:—

Percentage of fat (ether extract) in basal diet	...	0·09
Percentage of true neutral fat	approx.	0·06
Average weight of daily ration consumed ...	...	15 g.
Daily fat intake in basal diet ...	...	9 mg.
Ether extract of 5 c.c. carrot fraction (mainly lipoid matter and pigments, less than half neutral fat)	...	11 mg.
Approximate daily intake of neutral fat	...	14 mg.

Unless this minute amount of fat plays as important a rôle in the metabolism of the organism as do the minute quantities of such substances as the accessory factors, it is reasonable to suggest that pure fats are dispensable constituents of the mammalian diet. This does not, however, diminish the value of fats in the food, for one is led to think that the subnormal growth observed in these experiments is largely due to the difficulty of balancing the protein level and calorific intake on a diet containing no neutral fats.

The isolation of proteins from leaves. By A. C. Chibnall and S. B. Schryver. (*Preliminary Note.*)

Although proteins have been detected by microscopic methods in leaves, very little is known of their properties, and no one has, until recently, succeeded in isolating them from the plant tissues. Within the last week or so a pamphlet has been received from Professors Osborne and Mendel (*Year Book*, No. 18 of the Carnegie Institute for 1919, pp. 352–360) in which, in a couple of short paragraphs, they give a preliminary account of the isolation of proteins from spinach leaves by boiling the dried material with 60 p.c. alcohol containing 0·2 p.c. potassium hydroxide.

We have been engaged for some time on similar investigations and have now available considerable quantities of complex nitrogenous substances obtained from cabbage leaves, but by a method fundamentally different from that described by Osborne and Mendel in the pamphlet just referred to.

The work was started in 1916 by one of us (S. B. S.) in conjunction with Miss Laura M. Saunders, but had to be abandoned after some months owing to the pressure of other duties. It was re-started by us last winter and has since been continued without intermittence, and we availed ourselves of the preliminary results obtained by Miss Saunders.

The ultimate object of the research was the investigation of the nitrogenous metabolism of leaves, and the immediate object the separation and isolation of the various groups of nitrogenous substances. Cabbages were chosen, not because they form ideal material for the work, but because they were obtainable in quantity in London at the time the research was started.

The method employed was to treat the disintegrated material with water containing a cytolytic agent and, as a result of the quantitative work of Miss Saunders, water saturated with ether was employed. The extract made by this solution, after pressing from the cabbage residues, was opalescent, and after evaporating off the ether in a current of air, slowly deposited a flocculent precipitate. The precipitate formed very quickly, however, on warming gently (from 40°–60° C.). It rapidly settled and the clear liquid could be easily decanted off. The precipitate, which was found to consist chiefly of complex nitrogenous substances, was of greenish colour, and was drained on folded filters and washed with graded strengths of alcohol, then ether and finally dried. An amount of lipoids and green pigment could be extracted from the powder thus obtained by ether in a Soxhlet apparatus. After this treatment, it was of greyish colour and contained about 11 p.c. of nitrogen. About 200 grams were obtained from 70 kilos of the soft parts of the leaves (from which the coarser mid-ribs had been separated) which were got from 135 cabbages.

On further examination the powder was found to contain small amounts of substances soluble in water (apparently carried down from the main bulk of the fluid by adsorption) and the remainder could be separated into two approximately equal portions, one of which was soluble in dilute alkalis, and the other insoluble in solvents. The latter, although admixed with a certain amount of mineral matter, was found to contain 12 p.c. nitrogen and only very small traces of phosphorus. From the alkaline solution an amorphous acid could be precipitated by mineral acids. This contained 11 p.c. nitrogen and 0·7 p.c. phosphorus, and possibly contains a small amount of nucleoprotein. Products of this character are probably widely distributed in plant tissues and similar substances have been obtained by the same methods from pea-pods (both the acid and insoluble substances), although in this case they were admixed with appreciable amounts of starch, from which no doubt it will be possible to separate them by means of diastase.

The principle involved in the separations described above has, so far as we know, been employed only, if at all, to a very limited extent. Products insoluble in water have been separated from the tissues, by obtaining them in a colloidally dispersed form, and subsequent flocculation. This result has been attained in the case mentioned above by lowering the surface tension of water by ether. It may be possible to stabilise the colloid further by the addition of salts containing multivalent ions with an opposite charge, and to follow by flocculation methods usually employed in such systems. Researches are now proceeding with the object of developing this method, and of characterising more fully the products already obtained.

PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY,

October 16, 1920.

The liberation of CO_2 from carbonate by blood and serum.

By E. F. ADOLPH.

It was demonstrated by Setschenow(1) in 1859 that nearly all the CO_2 could be pumped from blood without the addition of acid. A few years later Pflüger(2) showed not only that all the CO_2 could be removed by an efficient pump, but that blood would even liberate CO_2 from sodium carbonate added to it. Buckmaster(3) has repeated Pflüger's experiment, with a negative result.

Dr J. S. Haldane suggested an easy method of testing Pflüger's observation, without the use of the blood-pump. An air current, freed of all traces of CO_2 by passage through a pair of soda-lime tubes, is bubbled through blood, and finally through a tube containing baryta solution. The tube containing blood is immersed in a water bath at 40°; amyl alcohol is added to inhibit foaming; and the stream of air is driven through the apparatus by a water-pump at the rate of about 400 c.c. per minute. Under these conditions no more CO_2 is driven from the blood into the baryta solution after about 30 minutes. If acid be added to the blood at this time, there is no CO_2 evolved, indicating that Pflüger's statement concerning the complete removal of CO_2 without adding acid is correct. The aeration is now interrupted, and sodium carbonate solution is introduced into the blood tube without allowing room air to enter. In the first minute after resuming aeration a fresh baryta solution is well clouded by CO_2 coming from the blood mixture. CO_2 continues to be evolved for an indefinite time, with gradual diminution in the rate of its production.

It is well known that sodium carbonate alone will not yield CO_2 without the addition of acid. This was confirmed by experiments in which carbonate alone was aerated in place of blood. In several trials the apparatus was modified so that carbonate as well as blood was aerated before mixing.

By the aeration method the demonstration has been repeated four times on defibrinated blood from three men, and six times on blood from two different sheep. The carbonate may be added in almost any concentration, but a high concentration gives a small yield of CO_2. The best results were given by adding to 10 c.c. of blood, from 0·02 to 0·05 gm. of Na_2CO_3 dissolved in 5 c.c. of water free from CO_2.

Reduced blood also liberates CO_2 from carbonate. This was demonstrated by using a stream of hydrogen gas in place of air.

On substituting serum for defibrinated blood, the demonstration became more difficult. Pflüger(2) was never able to pump out all the CO_2 already present in serum. According to Moore(4), however, it is possible to remove all the CO_2 by prolonged aeration. By aerating sheep serum in our apparatus for over an hour the removal of CO_2 was too incomplete to give a definite result upon the addition of carbonate. Sertoli(5) showed that other protein solutions, namely, lens globulin and serum globulin, would decompose sodium carbonate in a vacuum.

In criticism of Buckmaster's performance of the Pflüger experiment, in which he used a mercury pump, it may be suggested that he seems to have failed to boil the mixture of blood and carbonate in the vacuum. Any carbonic acid formed will remain in solution until extracted by some vigorous means, such as boiling, shaking, or bubbling gas through the mixture.

The result of these experiments should clear up much of the misunderstanding which has arisen in recent years concerning the mode of combination of CO_2 in blood. It seems clear, as Zuntz(6) argued forty years ago, that the whole of the CO_2 chemically combined in the blood of the living body is present as bicarbonate.

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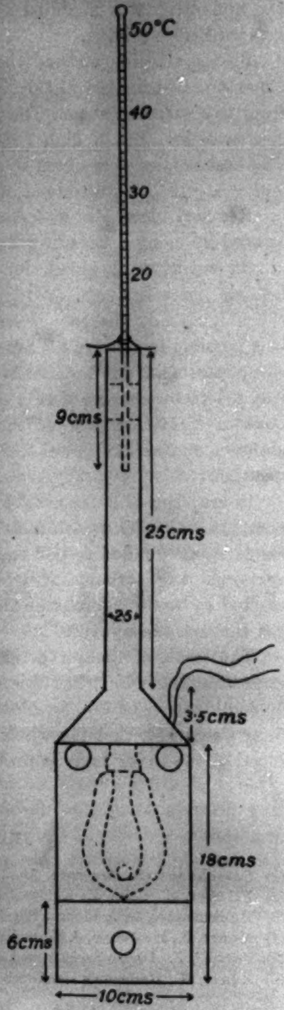
The ventilation thermometer or comfimeter. By Leonard Hill.

In order to keep comfortable conditions in rooms by determining the cooling power by convection or radiation—convection depending on the temperature and movement of the air—I have designed an instrument, which I call a "Comfimeter." This takes the place of the Katathermometer for ordinary civilian use. The Katathermometer requires to be heated in water, and the cooling rate measured as the meniscus drops from 100° to 95° F. The warming of the Katathermometer and the time measurements are against its popular use. The Comfimeter is an instrument which can be read at any time. just as the ordinary dry bulb thermometer; it consists of a cylindrical metal box 18 cm. high and 10 cm. in diameter, in which is inserted an 8 candle-power carbon-filament lamp, the lower part of the box being removable for this purpose. On the top of the box is fixed a metal cone, which in its turn forms a union with a chimney 25 cm. long and 2½ cm. in diameter. There are some holes in the lower and upper part of the box for purposes of ventilation. An ordinary dry bulb thermometer is introduced into the chimney so that the bulb hangs within it to a depth of 9 cm. The thermometer rests on the top of a chimney by means of a wire which is twisted round its stem in a suitable fashion. There are two discs of gauze through which the stem of the thermometer passes to give it a central position within the chimney. The 8 candle-power



lamp acts as a constant source of heat and the box and the chimney are cooled by radiation and convection.

Under satisfactory conditions on ideal summer days with the windows open the Comfimeter standing on the table in my room indicates a temperature of about 25° C. In a close room heated with hot water coils on and the windows shut the Comfimeter indicated a temperature of 40° C. If the Comfimeter be sheltered from wind by a screen of cotton material placed around it, it will indicate in place of say 30° C. a temperature of 45° to 50° C., while the dry bulb thermometer, standing within the same screen will only vary a degree or two as the result of screening. These figures show how sensitive the Comfimeter is to the cooling power of the wind. It must of course be given time to get in equilibrium with the environmental conditions.

When the Comfimeter indicates 30° C. the Katathermometer gives a cooling power of about 7 millicalories per square centimetre per second. So long as schools and factories are kept with the Comfimeter indicating somewhere about 30° C. fresh conditions suitable for work will be obtained.

The instrument is made by Siebe German, Ltd., 187 Westminster Bridge Rd., S.E.

Formation of a new "Compressor Urethræ" muscle in a boy who suffered from incontinence due to congenital defects.

By Ralph Thompson.

S.W., well developed male subject, æt. 16, was admitted to Guy's Hospital suffering from incontinence of urine.

The following anatomical conditions were present:

(1) A deformed penis with no true urethra.

(2) A circular opening situated upon the dorsum of the penis. This opening was constantly open, and urine was continually dribbling away through it.

(3) The pubes were not united across the middle line, but were separated by a distance of more than one inch.

(4) There was a bladder capable of holding not more than five ounces of fluid, and which could be entered through the hole described under para. (2).

The condition, therefore, was one of epispadias, or congenital penile fistula. This latter term is preferable as "epispadias" implies a comparison with "hypospadias." There is in fact no comparability between the two conditions.

The failure on the part of the pubes to unite in the middle line points to a real connection between epispadias and "ectopia vesicae."

The problem was to provide a compressor muscle in order to prevent the constant passage of urine, which had led to the boy being a constant inmate of public institutions. There was a possibility of finding a perineal compressor, and this received consideration but the anatomical conditions, forming the foundation of such a surgical procedure, were not considered favourable.

The possibility of a portion of the rectus abdominis muscle being used as a compressor was considered and the following facts as regards its anatomy were elicited.

1. *Nerve supply.* The lower two-thirds of that portion of the muscle, which lies below the navel, was found to be supplied by fibres coming along the twelfth dorsal nerve, which splits into two branches and enters the outer border of the muscle one branch proceeding upwards, and one downwards.

2. *Vascular supply.* The deep epigastric artery passes obliquely upwards and inwards on the posterior surface of the rectus muscle to which it is adherent and gives off, as it proceeds, segmental branches, which lie fairly near each other.

3. *Position.* The rectus muscle lay near the urinary orifice and could be turned downwards with the least possible interference with its functions as a possible compressor.

OPERATION.

The lower two-thirds of that part of the right rectus abdominis which lies below the navel, was divided from the upper part. No effort was made to see the twelfth dorsal nerve, or the insertion of the muscle into the pubes. The deep epigastric artery was divided and ligatured. The lower parts of the muscle, and artery, were then turned downwards and the (true) upper end of the muscle was split longitudinally for 1½ inches. The split muscle was then laid round the urinary orifice in a special bed prepared for it—the ends of the muscle were sutured to each other and to the tissues on the underside of the urinary orifice. The wound was then closed up.

The operation was performed on 5th June, 1920. Patient was holding his water all night, approximately about 26th June, *i.e.* three weeks after the operation; but could not exercise much control over the flow of urine during the day—in fact there was very little if any control.

He was instructed to bend his head forcibly forwards when he desired to hold his water, and on 16th July he held his water, standing up or walking about for 1½ hours. On 18th July he held his water for 1½ hours and passed it in my presence. There was a good stream.

On 26th July he held his water for 3 hours.

AFTER HISTORY.

Patient continued to hold his water well. He entered my service and did his work well. There is a slight forward inclination of the body upon the thighs when he walks, and I noticed that he preferred to stand rather than sit about. This peculiar gait may however be due to pain associated with the formation of a right renal calculus for which he was re-admitted to Guy's Hospital, and the stone was removed.

The right kidney was very small and pale, but it was not removed.

Date of operation for Nephrolithotomy, 10th September.

Before the operation on this date he had incontinence, and although patients with assumed normal nervous conditions do suffer from incontinence of urine as a symptom of renal calculus, in this particular case the incontinence may be associated perhaps with the fact that the fibres which control the patient's compressor come down along a nerve which is intimately associated with the kidney.

For some little time after the second operation in Guy's Hospital the patient had incontinence but on the 28th September the sister of the ward reports that he is holding his water well, and does not wet his clothes.

Note on the sense of smell. By H. HARTRIDGE.

An experiment frequently quoted (1), (2) as showing that the air must be in motion in order that the sensation of smell should be aroused is one in which an unstoppered bottle of an aqueous solution of ammonia is held near the anterior nares, the posterior nares being closed or the breath being held so that no current of air is caused to circulate past the olfactory epithelium.

Under these circumstances the sting of the ammonia gas is strongly felt by the nasal mucous membrane, but the smell of the gas is not perceived. But if now the bottle be removed and the experimenter holding his nose walks into another room, and an inspiration be then made through the nose, immediately a powerful smell of ammonia is recognised.

It would seem that the conclusion drawn from this experiment that the air must be in motion past the olfactory epithelium in order that a smell should be perceived, is without a firm basis since an alternative and more plausible hypothesis is able to account for the facts: when the mouth of the bottle is first held near the nose the ammonia vapour enters the nasal cavity by diffusion and reaching the moist mucous membrane is immediately dissolved. As more enters, this also is dissolved, until the tension of the gas at the surface of the mucous membrane is equal to that in the air. When this is the case, the gas penetrates more deeply and thus reaches fresh mucous surface, where it also dissolves.

Now it is only when the whole area of mucous membrane between the anterior nares and the olfactory area is partially saturated, that any appreciable quantity of the gas reaches the olfactory mucous membrane itself. As a rule, long before this becomes possible, the nasal branch of the Vth nerve (which supplies the general mucous membrane of the nose with sensibility) has received stimuli so painful that the subject terminates the experiment. The reason for the gas not acting as an odour is not therefore because the air is not in motion, but because the gas goes into solution before it reaches the olfactory mucous membrane.

In the case of most odorous substances two further factors beside solubility act against their reaching the olfactory mucous membrane unless there is a definite air current: (1) their slow rate of diffusion, and (2) the fact that air containing them has a greater specific gravity than that of pure air. Thus, if the experiment be tried with chloroform, the vapour can be felt trickling along the floor of the nasal fossal without the smell of chloroform being observed.

There is a further experiment that at first sight is at variance with the solution hypotheses outlined above. Namely, that if the nasal cavity be filled with the vapour of an odorous substance and the movement of the vapour be then stopped suddenly by either pinching the nose, or by voluntarily blocking the posterior nares, then the sensation of smell rapidly wanes. It might be thought that the cessation of movement of the air over the olfactory mucous membrane was responsible for the disappearance of the sensation. It is more likely, however, that in this case also it is the rapid solution of the odorous particles from the air as they come into contact with the membranes of the nasal cavity, coupled with the using up of the molecules of the odorous substance already dissolved by the olfactory mucous surface (since in order to arouse a smell, presumably a certain quantity of the odorous substance is expended in order to bring about those chemical (or physical) changes which are at the basis of the sensation of smell). A further factor in the cessation of the stimulus may be accommodation on the part of the olfactory epithelium to the static conditions that now exist there.

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(2) Greenwood. Physiology of the Special Senses, p. 42. 1910.

Surface tensions of oil-water interfaces. By H. Hartridge and R. A. Peters. (*Preliminary Communication.*)

We have found that the surface tension developed at the interface between an oil and water varies with the hydrogen ion concentration of the water. This is shown in the photograph (Fig. 1). When the H ion concentration of the fluid is low (acid), the surface tension is high and *vice versa*. Near the neutral point, using olive oil, there is a fall in the surface tension of about 35 % for a change of $P_H = 1{\cdot}0$. The experiments have been performed by both the capillary height and drop weight methods, which gave concordant results. In the case of paraffin and

water, no such fall was observed, and in the case of benzene the fall was variable and very small.

It has seemed to us that oils largely consisting of pure esters, *e.g.* specially treated olive oil, castor oil, nut oil, cod-liver oil gave the largest and most constant variations with P_H. On the contrary oils containing soaps or fatty acids as impurities gave values of surface tension, which decrease rapidly as time goes on. This fact was confirmed by measuring the interfacial tension with olive oil to which oleic acid or soap had been added. A satisfactory method of removing these bodies for the purpose of surface tension determinations was boiling for a number of hours in contact with a considerable excess of frequently changed tap-water. Temperatures between 20° C. and 40° C. were found to have little effect upon the interfacial tension at the water olive oil interface. Further experiments are in progress, as it is hoped to develop the above method into an accurate technique for estimating hydrogen ion concentrations, of physiological liquids.

The striking difference between the behaviour of a benzene-water and oil-water system to changes in the reaction of the water, seems to be in support of the views upon the chemical nature of surface forces, originally proposed by Hardy(1), and subsequently developed by Langmuir(2) and Harkins(3). It is also in keeping with Langmuir's observations upon the effects of acid and alkali upon films of oil and fatty acid spread upon water.

Note. Harkins, Davies and Clark remark that the change of surface tension at a benzene-water interface is too small to account for movements of muscles. The argument however does not seem to us valid. Fats and fatty acids are constant constituents of tissues, and our experiments indicate that with such systems as these, considerable changes in surface forces occur when the reaction changes.

(1) **Hardy.** Proc. Roy. Soc. 88 A. p. 303. 1913.
(2) **Langmuir.** J. Am. Chem. Soc. 39. p. 1883. 1917.
(3) **Harkins, Davies and Clark.** J. Am. Chem. Soc. 39. p. 541. 1917.

The method of mixtures as applied to the calibration of instruments for measuring the CO in blood. By H. HARTRIDGE.

The method of mixtures first used by Haldane and Smith(1) for calibrating the carmine method, has recently been employed by van Slyke and Salvesen(2) for testing the accuracy of their gas analysis method of estimating CO in blood.

In the method of mixtures a quantity of well-stirred defibrinated blood is divided roughly into two parts. One of them is well shaken with air, the other with CO gas. Various mixtures of measured volumes of these two liquids are now prepared, the assumption being made that the mixtures will have a CO saturation that can be calculated from the amounts of the two constituents. Such is not the case, however, because when blood is shaken with CO gas not only does a part combine with (and if enough CO be used and shaking continued long enough) completely saturate the hæmoglobin but also a further variable and unknown amount of the gas dissolves in the plasma. (Experiments are also to be published later which show that CO combines with the plasma proteins. The amount is not large however.) The full amount of CO in solution in plasma at N.T.P. is about 3 % per volume, so that a mixture of equal parts of blood saturated with air and CO (which van Slyke assumed to contain hæmoglobin 50 % saturated with CO) is found by calculation (and experiment confirms the value) actually to contain 57·5 % CO Hb, owing to the fact that the CO in solution has itself displaced the oxygen from 7·5 c.c. of the blood shaken with air. The differences between the calculated and the actual CO saturations for other mixtures are given in the following table:

% blood in mixture initially saturated with CO	Calculated % CO saturation	Actual % CO saturation	Difference
100	100	100	0
75	75	86·25	11·25
50	50	57·5	7·5
40	40	46	6
30	30	34·5	4·5
20	20	23	3
10	10	11·5	1·5
0	0	0	0

It will be seen that the differences are considerable.

Now the figures in the last column could be used for correcting the calculated % if it were not for the fact that it is very difficult to bring a quantity of whole blood into equilibrium with CO even at a high tension. The reason for this appears to be that the complete solution of CO in the plasma is much less readily effected than the complete saturation of the hæmoglobin with the gas.

In my opinion the method of mixtures is unreliable even when the corrections indicated are applied to it, as a rule it would appear to be better to devise some other method which does not suffer from the same inaccuracies (*e.g.* the double wedge trough method(3)), or to compare the values given by the method of estimating CO which is under test with some standard method of known reliability such as the blood gas-pump.

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(2) van Slyke and Salvesen. Journ. Biol. Chem. 40. p. 103. 1919.
(3) This Journal, 44. p. 1. 1912.

Observations made by means of the fractional method of gastric analysis. By T. IZOD BENNETT, with the assistance of Messrs P. McG. MOFFATT, MITCHELL, A. T. W. POWELL and others. (*Preliminary Communication.*)

Some seventy normal men have now been investigated by this method, pathological cases being excluded by clinical examination, and many of my results have been checked and elaborated by means of radiographic examination. This latter part of the work has been carried out by Dr P. J. Briggs.

Dr J. A. Ryle has made many parallel observations on the pathological side; and Dr J. F. Venables has helped me with the hypnotic portion of the research.

The following are our chief results:

(1) In normal healthy men curves of gastric acidity can be obtained which include those types which the pathologists have called *Hyperchlorhydria*, *Hypersecretion* and *Achlorhydria*.

(2) At least four groups of normals can be distinguished:

(*a*) Those whose free acidity exceed 50 c.c. of *N*/10 NaOH %.
(*b*) Those whose free acidity is below 20 c.c of *N*/10 NaOH %.
(*c*) An average intermediate group.
(*d*) A group of average acidity but with very rapidly emptying stomachs.

(3) The psychic secretion of gastric juice in man is so slight that I have been unable to demonstrate it; hypnosis, the exhibition of food, and actual tasting and chewing of food being without effect.

(4) A definite secretion of HCl can often be provoked by water, whether swallowed or injected directly into the stomach.

(5) The emotions of fear and nausea can by this method be demonstrated to produce strong sympathetic inhibition of secretion and motility.

(6) The idea of hunger can be demonstrated to produce rapid emptying of the stomach; whether it also excites secretion I cannot definitely say.

(7) Any stimulus which, whilst gastric secretion is in progress, causes the stomach to empty more rapidly, must thereby cause an increase in the acidity of the gastric contents.

(8) Massage of the abdomen, as usually performed, delays, rather than quickens, the emptying of the stomach.

(9) The majority of human stomachs are never empty between the hours of 8.30 A.M. and 1.30 A.M.

(10) Bile is present in the gastric contents of a large number of human subjects at frequent intervals during the day.

(11) The vagal influence on the secretion of gastric HCl is demonstrated by the effect of atropine whether given hypodermically or as a wash before the meal.

(12) Pilocarpine increases motility, but I have not been able to demonstrate that it increases gastric secretion; this may be due to the excessive salivation produced which tends to dilute the gastric contents.

An electrically heated kata-thermometer. By LEONARD HILL and D. HARGOOD-ASH.

The electrically heated kata-thermometer is a modified form of the ordinary "kata," the heat being, in this case, supplied by passing a known current through a small coil of high resistance wire fused into the bulb of the "kata" by means of platinum leads. The bulb is the usual size and shape but the stem is made long enough to allow of a calibration from 28° to 44° C.

In the ordinary "kata" the heat loss per second, H, varies with θ, the difference of temperature between the mean temperature of the range of cooling and the air temperature, according to the equation:

$$H = (\cdot 27 + \cdot 49\sqrt{v})\,\theta$$

where v is the wind velocity[1].

If a steady current is passed through the coil of the electrical "kata," a constant amount of heat will be supplied per second and when the "kata" reading is constant, it follows that the heat loss is also constant and equal to the supply. That is to say that the "kata" reading gives the mean temperature of that range of cooling which the "kata" would have to cool through if it were to lose, under existing external conditions, that amount of heat which it receives, and, if this is known, the heat loss over the usual "kata" range (100–95° F.) may be determined.

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[1] A more extended investigation of winds has given us the formula $H = (\cdot 12 + \cdot 54\sqrt{v})\,\theta$ as holding over the range of velocities particularly the lower ones which were not previously investigated.

The instrument then is calibrated over a range of temperature from 28° to 44° C., the current being chosen so that when the reading is 36°·5 C. the heat loss is 6 millicalories per square cm. per second. The range of cooling powers of the instrument is approximately from 4 to 9. By using currents of different strengths, however, a variety of ranges may be obtained for the same instrument.

The equation connecting the cooling power H and the "kata" reading ϕ is deduced as follows:

The ordinary equation for still air is

$$H = \cdot 27\,(\phi - t) \quad . \quad . \quad . \quad . \quad . \qquad \text{(i)}$$

where H is the heat loss in millicalories per sq. cm. per sec., ϕ = the mean temperature of range of cooling (in the ordinary kata), and "t" the temperature of surroundings in still air.

If for a cooling power of "6" the meniscus of the electrical "kata" stand at 36°·5 C., then in the above equation $\phi = 36{\cdot}5$ and we have

$$\begin{aligned} 6 &= \cdot 27\,(36{\cdot}5 - t) \\ \text{or} \qquad t &= 36{\cdot}5 - 6/{\cdot}27 \\ &= 36{\cdot}5 - 22{\cdot}2 \\ &= 14{\cdot}3, \end{aligned}$$

i.e., if the still air temperature is 14·3, the current must be adjusted so that the meniscus stands at 36·5. If, however, the still air temperature is some other value, say t_0, then since the heat loss must be the same as before, *i.e.*, 6 millicalories per sq. cm. per sec., by substituting the value t_0 in equation (i) we obtain the value of ϕ

$$\text{or} \qquad \left.\begin{aligned} 6 &= \cdot 27\,(\phi - t_0) \\ \phi &= 6/{\cdot}27 + t_0 \\ &= 22{\cdot}2 + t_0 \end{aligned}\right\} \quad . \quad . \quad . \quad . \quad . \qquad \text{(ii)}$$

and this value of ϕ is the temperature at which the meniscus will stand at an air temperature t_0, if the current is such that it would stand at 36·5 with an air temperature of 14·3.

Having in this way determined the constant current required it is necessary to know what cooling power is indicated by any position of the meniscus, *i.e.*, for any value of ϕ. Let the observed value be ϕ_1, then in the still air chamber it would have given this reading if the temperature t_1 had been given by

$$t_1 = \phi_1 - 22{\cdot}2 \quad . \quad . \quad . \quad . \qquad \text{(iii)}$$

obtained from equation (ii), and the kata cooling power in still air at temp. t_1, expressed in terms of cooling over the normal range is given by

$$\left.\begin{aligned} H_1 &= {\cdot}27\,(36{\cdot}5 - t_1) \\ &= {\cdot}27\,(36{\cdot}5 - \phi_1 + 22{\cdot}2) \text{ substituting from (iii)} \\ &= {\cdot}27\,(58{\cdot}7 - \phi_1) \end{aligned}\right\} \quad . \qquad \text{(iv)}$$

therefore the cooling power is known when the value of ϕ_1 is observed. This relation between H and ϕ being linear, a straight line graph may be plotted and the cooling power read off directly.

The instrument is made by Mr J. Hicks, 8 Hatton Garden, E.C.

A clinical method for the quantitative determination of the creatinine-creatine content of urine. By David Burns.

As in recent years the quantitative determination of creatine and creatinine has assumed clinical importance, the following simple method which has been used with success by some 1200 students and which involves the provision of no expensive apparatus, was devised for ordinary medical class and clinical work.

Apparatus for Creatinine Estimation:

Haldane (or Sahli) hæmoglobinometer graduated tubes of about 8 mm. diam.

Flask or cylinder with a capacity of over 250 c.c. with a mark at 250 c.c.

Pipettes, 5 c.c., 10 c.c., 15 c.c.

Capillary dropping pipette (ungraduated).

Solutions:

Standard solution of potassium bichromate, 0·5 N.

Approx. 10 % sodium hydrate solution.

Saturated picric acid in water.

Method. Pipette 10 c.c. of urine into the 250 c.c. flask, add 5 c.c. 10 % NaOH and 15 c.c. sat. picric acid. Mix and allow to stand for 5 to 7 minutes. Dilute with water up to the 250 c.c. mark and mix thoroughly. Fill one of the graduated Hb tubes with the standard bichromate solution. With the capillary pipette, run the urine-picric acid-soda mixture into the other graduated Hb tube up to the 50 mark. Proceed now as in the estimation of Hb, adding water drop by drop and mixing till the colour of the mixture is just a shade darker than the standard (1st reading). Add drops of water and mix till the colour is just too light (2nd reading). *The mean of these two readings gives the amount of creatinine in milligrams per* 100 *c.c. of urine.*

Theory. Folin's argument was that, as 8·1 mm. of the bichromate gives a colour equivalent to that produced by a creatinine-picric acid-soda mixture containing 10 mgs. in 500 c.c. and, as the colour of the unknown matches the colour of the standard bichromate, the concentration of creatinine in the unknown must be proportional to the concentration of the standard in the ratio of the readings. That is, $10 \times \frac{8{\cdot}1}{R}$ = mg. in 10 c.c. urine (diluted to 500 c.c.) where R is the reading in a Duboscq colorimeter.

The width of the tubes in this method is constant but the dilution varies first of all by ½ (250 as against 500) and then by $\frac{\text{(the reading)}}{50}$. Therefore, to get the *percentage* amount when the reading is 66 one modifies the Folin formulæ as follows:

$$\frac{10}{1} \times \frac{10}{1} \times \frac{8{\cdot}1}{8{\cdot}1} \times \frac{250}{500} \times \frac{66}{50} = 66 \text{ mg.}$$

It is immaterial what the width of the two Hb tubes is provided (1) that it is the same in both tubes and (2) that it does not differ much from 8 mm.

Results:

Class of 40—working in pairs.

Number		Reading	Number		Reading
4	read	62	6	read	67
3	„	63	1	„	69
3	„	65	1	„	72
1	„	66	1	„	75

Half the class got results which are close to the average value, viz.: 65·8. The reading given in a Duboscq colorimeter was 66. This was a first attempt by a medical class.

Creatine, after conversion into creatinine in the ordinary way, may also be estimated by this method.

Arterial CO_2 tensions. By J. M. H. Campbell (Hilda and Ronald Poulton Fellow at Guy's Hospital), and E. P. Poulton (Beit Memorial Research Fellow).

The method of arterial puncture(1) has made it possible to measure the volumes of oxygen and carbon dioxide in arterial blood. But the more important factor in the case of CO_2 is the partial pressure of the gas and there is no simple method of measuring this directly. The CO_2 dissociation curve varies to a moderate degree in different persons, and so it is necessary to draw the dissociation curve in each case, to be able to infer the CO_2 partial pressure. This was done by the method which has been fully described(2)—the blood, with oxalate up to ·5 per cent., being exposed for fifteen minutes at 38° C. in tonometers to various pressures of carbon dioxide. The alveolar air was taken by a modification of the Hasselbalch-Lindhard method(3). The valves are watched carefully and the patient ordered to expire deeply either at the end of inspiration or expiration, a sample being taken during the last part of the forced expiration. The results are shown in the two tables (I) cases without breathlessness and (II) cases with breathlessness.

Table I.

	Age	Vols. CO_2 in 100 c.c. arterial blood c.c.	Vols. CO_2 in 100 c.c. blood at 40 mm. c.c.	CO_2 pressure in art. blood mm.	CO_2 pressure in alveolar air		
					(1) average mm.	(2) inspiratory mm.	(3) expiratory mm.
12. Fractured patella ...	43	52·6	56·0	34	38·6	36·7	40·5
13. Acromegaly	28	52·8	53·5	39	39·6	—	—
14. Chronic otitis media ...	26	52·6	55·5	36	37·4	36·3	38·5
15. Asthma after recovery from attack	50	52·5	54·0	37·5	37·3	36·0	38·6
8. Convalescent empyæma (1)	39	56·0	60·5	34*–37†	37·4	33·8	40·9
(2)	—	55·5	58·5	36	33·1	32·2	34·0
5. Polycythæmia	45	33·1	38·5	33·5	—	—	31·5

Table II.

3. Uræmia	39	36·7	37·5	36	—	—	27·0
1. Cardio-renal disease with severe hyperpnœa	52	57·1	50·5	56	—	—	31·2
9. Cardio-renal disease with moderate hyperpnœa	51	47·6	46·0	42·5	26·2	23·9	28·5
10. Cardio-renal disease with moderate hyperpnœa (1)	55	52·0	51·0	43 ‡	29·7	26·8	32·6
(2)	—	50·6	52·5	40 ‡	—	—	—
2. Valvular disease of the heart	21	54·9	52·0	46	—	—	31·3
4. Ditto	26	48·8	48·0	42	—	—	25·4
11. Ditto ... ... (1)	32	51·4	49·0	43	39·9	38·2	41·6
(2)	—	49·8	47·5	40	—	—	—

* Found by the usual method from CO_2 dissociation curve of blood.

† Found from CO_2 dissociation curve of true plasma.

‡ Taken later during Cheyne-Stokes breathing.

As regards the cases without breathlessness the alveolar CO_2 values correspond with the CO_2 pressure of the arterial blood within a few millimetres, agreeing with the results obtained experimentally by **Krogh** (4) and justifying the assumption which has usually been made in the case of man. Case 5 shows some diminution of the alkali reserve, since the CO_2 content at 40 mm. is diminished.

In the cases with breathlessness the mean alveolar CO_2 pressure was always less than the arterial CO_2 pressure. This was most marked in cases of valvular disease of the heart and cardio-renal disease. The uræmic case showed lowered alkali reserve (CO_2 37·5 % at 40 mm.) but this was not present in any of the cardiac cases. The arterial CO_2 in the uræmic case was low owing to the acidosis. In the cardiac cases the arterial CO_2 was higher than in the cases without breathlessness. In case 1 the value 56 mm. is remarkably high. He was obviously very breathless even when sitting at rest.

It would seem permissible to conclude that in cardiac cases of various types, one factor in the breathlessness is the increased tension of carbon dioxide in the arterial blood.

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Nutrition of the protozoa. By R. A. Peters.

2. *The carbon and nitrogen compounds needed for the growth of paramœcium.*

A continuation of the work upon the metabolism of a variety of the ciliate protazoon colpidium[1] has brought to light some rather interesting new features.

Technique. The technique described previously has not been modified. The experiments have been done throughout with the colpidium grown from a single individual isolated last November. Subcultures have been made in the ordinary bacteriological way by transferring one drop of a growing culture to a fresh tube. A substance has not been considered sufficient for growth unless it was found possible to subculture the organism successfully through 3–4 subcultures.

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[1] The ciliate organism resembling paramœcium has now been identified as colpidium.

As would be expected it has been found that in the absence of phosphate no growth would take place.

Ammonium glycerophosphate (Kahlbaum) has been found to serve as a complete source of nitrogen, carbon, and phosphorus. Ammonium glycerate plus ammonium phosphate will also serve in a similar way. Nitrogen therefore need not be in a more elaborate form than ammonia.

The table below is a summary of the results of substituting various organic bodies in the place of the glucose, amino acids, and lactic acid forming the carbon source of the previous medium[1].

Source of carbon	Growth	Source of carbon	Growth
Carbonate	Nil	Citrate	Nil
Formate	"	Tartrate	+
Oxalate	"	Glucose + lactate	+
Glycollate	"	Leucine (synthetic)	+
Glycerate	+		
Glycerophosphate	+		

It is quite possible that there may be a means of making colpidia live upon formates, oxalates or glycocollate, but the results as they stand show that these protozoa are not dependant upon complicated chemical bodies for their growth. They show a remarkable independence. This is also the conclusion reached from some experiments performed upon the effect of varying some of the inorganic salts. The organism for instance seems to be widely independent of extensive variation in the ratio between potassium and calcium salts, though apparently potassium is essential for growth.

The text-book statement[2] that "the animal organism, even in its lowest forms, the protozoa, is satisfied with nothing less complex than glucose as a source of carbon," is not true.

The effect of substituting uranium for potassium in growth media. By R. A. Peters. (*Preliminary Communication.*)

Potassium has been found to be essential for the growth of the particular species of colpidium described in a former paper.

Since Zwaardemaker (1) has stated that uranium will replace potassium in maintaining the normal activity of the frog's heart beat, an attempt has been made to substitute uranium salts for potassium in the culture media of the colpidia. In this work it was found essential to use quartz tubes because enough potassium appears to be dissolved out of glass ones (even Jena) to invalidate the results. The criterion of the efficiency of a medium has been, whether subcultures can be continuously made. Potassium deficient cultures will not subculture. The addition of uranium sometimes slightly improved the condition of the first subculture, subsequent subcultures dying out. When both uranium and potassium were present, the result seemed to be better, than with potassium alone. This accords with the observations of Sasaki(4) and others upon the beneficial effect of traces of uranium in bacterial cultures.

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[1] *J. Phys. Soc. (Proc.)*, 53. p. cviii. 1920.

[2] See Bayliss, *Principles of Gen. Phys.* p. 248.

The fact that uranium will not replace potassium in the growth process of these organisms is a biological case, in which the sole effect of potassium does not seem to be due to its radio activity. A. J. Clark(2) has recently stated that in the case of the frog's heart, uranium will not replace potassium in the same way in which rubidium will do so. The results indicate that if the effect of potassium is a radio active one (as claimed by Zwaardemaker), potassium can serve a double function in the organism. The improving effect of uranium salts upon growth may probably be correlated with the effect observed by Redfield(3) of small doses of β rays upon the division of *Nervis* eggs.

[I am indebted to my assistant H. Mowll for his valuable help in these experiments.]

[The expenses have been defrayed by a grant from the Government Grant Committee of the Royal Society.]

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An electrical pulse recorder. By A. V. Hill.

If a very fine exposed wire mounted in a tube be made one of the arms of a Wheatstone's bridge it will be heated by the current and its resistance will rise. If air be blown upon it, it will be cooled and its resistance will fall: if the bridge was previously in balance a current will now run in the galvanometer. By using a string galvanometer and a wire of suitable size the arrangement can be made very sensitive and extremely rapid in its action. A small vulcanite funnel is connected to about a metre of thick rubber tubing and pressed firmly upon an artery or vein or the chest wall. The pulse or heart beat causes alternating puffs of air to travel down the tube. These pass across the little heated wire and cool it, and the degree of cooling is recorded on a photographic plate in the camera of the string galvanometer. It is better to use a metal fibre in the galvanometer, as with an external low resistance it is, for a given sensitivity, more rapid in its movements. Typical records (made on myself) are shown in Fig. 1. It is obvious from a study of these that the method has possibilities, and it is extremely simple to use in any laboratory or hospital possessing an electro-cardiograph. The original instrument was made for me by the Cambridge & Paul Instrument Company, to whom, and especially to Mr C. C. Mason, my heartiest thanks are due.

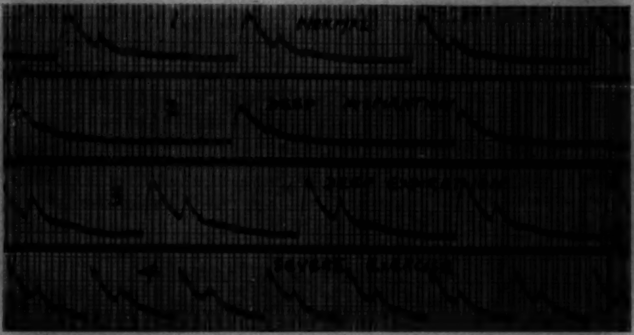


Fig. 1. To be read from left to right. Time in 1/25 sec. Metal fibre in galvanometer. (1) Normal; (2) deep inspiration; (3) deep expiration; (4) after skipping for one minute. Carotid artery.

The "tension-time" production of muscle. By A. V. HILL and W. HARTREE.

It is desirable to find some exact expression for the mechanical activity of muscles, some quantity which will cover all the phases of their contraction, and be more or less proportional to the chemical changes accompanying them. The work done by a muscle is not such a quantity, as (1) it depends on the manner in which the muscle is loaded and (2) it takes no account of the prolonged effort required to maintain a prolonged contraction. The tension developed in an isometric contraction is a much more fundamental quantity than the work done, but this also takes no account of the effort exerted in a prolonged contraction. A quantity however, which for lack of a better word we have called the "tension-time," can be shown to take account of the various factors, and also at a given temperature to be fairly accurately proportional to the total heat production of the muscle. In this quantity we believe we have found a generalised expression for the mechanical activity of muscle. Consider the curve made by a muscle in an isometric contraction recorded upon a uniformly rotating drum. The *total area* of this curve is what we have called the "tension-time" produced by the muscle in that contraction, and an experimental relation between

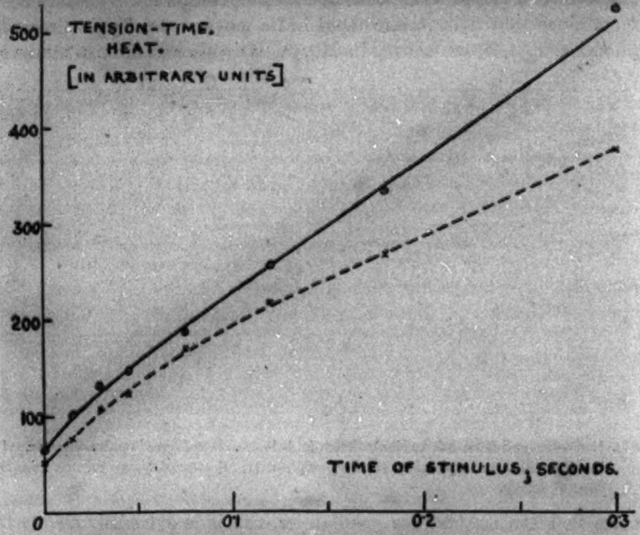


Fig. 1. *Continuous line.* Relation between "tension-time" production and duration of stimulus.
Dotted line. Relation between heat production and duration of stimulus. Sartorius muscle of frog. Isometric contractions. Temperature, 10° C.

NOTE.—Zero time of stimulus refers to the case of a single shock. In the other cases the stimulus was given by an alternating current at 90 periods per second.

the "tension-time" produced and the duration of the stimulus is shown in Fig. 1. In the same figure is shown the relation between the heat production and the duration of the stimulus. It is clear that the "tension-time" and the heat produced in a contraction are approximately proportional.

The "tension-time" production of a muscle is much more "efficient" at a low temperature than at a high one; the quantity

$$\frac{(\text{"tension-time" production})}{(\text{heat production})}$$

is decreased from 3 to 4 times by a rise of 10° C. This is of importance as a practical point in the life of a cold-blooded animal, and is of considerable intrinsic interest.

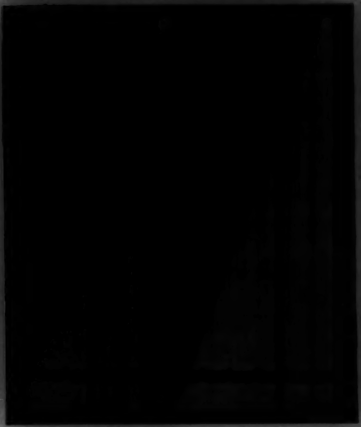
It is possible to express the conception of "tension-time" in a more intelligible form. If a muscle exert its force in pulling at a very heavy mass which it can barely move its contraction will remain practically isometric, while the *momentum* it produces in the mass can be shown to be equal to the "tension-time" exerted, defined as above. The momentum, *i.e.* the product of the mass and its velocity, can be measured by appropriate means, and it will be found that, at a given temperature, the momentum produced is proportional to the total heat production. We may say therefore that *the muscle is a machine for producing momentum*, the *momentum produced being proportional to the extent of the chemical changes accompanying contraction.*

This fact may be of importance not only in the case of the isolated muscle but also in that of experiments or observations on man. Instead of comparing the CO_2 given out with the work done, which depends on so many factors, it would be better to compare it with the total momentum produced, which is independent of all these factors (except temperature, which in man is constant). It would require suitable means for measurement, but this presents no fundamental difficulty.

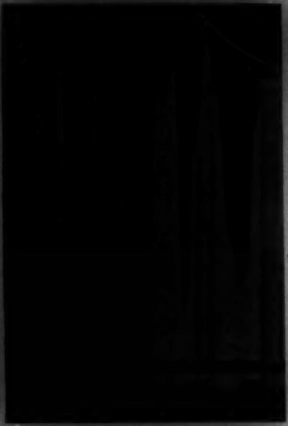
On the contractility of amputated parts of plants. By A. D. Waller.

At the June meeting of the Physiological Society, I demonstrated by experiment at a magnification of 1000 the fundamental distinction between the elongation caused by growth and that caused by turgor, the former being irreversible and increasing in a straight line during short periods of observation, the latter reversible, *i.e.* a temporary elongation followed by recovery (shortening).

The distinction was illustrated by the following graphs.



Blue Lupin Spike.
Elongation by growth (irreversible).
Rate = 70 μ in 5 min.
= 14 μ per minute.



Blue Lupin Spike.
Alterations of turgor (reversible) caused by strong electrical currents.

In the second graph the effects of electrical excitation, the incidence of which is shown on the signal line appear at first sight as if they were to be vegetable contractions analogous to muscular contractions, they are in reality temporary elongations due to temporary alterations of turgor, they are assuredly not pulsations of growth followed by "de-growth," similar to the up and down curves given in illustration of rates of growth in a recent publication[1]. In point of fact we may be well assured that an up and down movement cannot be a movement of growth (irreversible), but can only be a turgor movement (reversible).

The effects are exceedingly various—"tetanisation" produces all sorts of effects upon all sorts of materials living and non-living, and in the case of the amputated parts of plants it is always difficult to decide whether a given effect is physiological or not. Tetanisation of a plant causes elongation or shortening or elongation followed by shortening. Tetanisation of a damp fiddle string under similar conditions causes a closely similar elongation or a shortening followed by elongation[1]. We may not therefore without further proof admit the effects of the tetanisation of plants as evidence of physiological contractility, nor of a physiological modification of growth.

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[1] Bose and Das. *Proc. R. S. B.* vol. 90. 1899. Fig. 6, p. 37, record of a single growth pulse of crocus.

As regards the amputated (surviving) parts of plants we must conclude that

1. Movements of elongation visible at a magnification of 1000 and *a fortiore* at a magnification of 10^7 are not necessarily due to growth.
2. Movements of contraction visible at similar magnifications are not necessarily due to a vegetable contractility analogous with the contractility of animal muscle.

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[1] Waller. Demonstration of the contractility of nerve and of fiddle strings and other strings. *Proc. Physiol. Soc.* March 21, 1908. *J. of Physiol.*

PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY,

November 20, 1920.

Deglutition apnœa. By G. A. CLARK.

In experiments carried out to determine the duration of the reflex apnœa in deglutition, the respiratory movements were recorded by means of a stethograph and the rising of the larynx in swallowing by a double tambour over the upper part of the thyroid cartilage communicating with a writing tambour; a time-marker recording seconds was used.

185 observations were made on 17 individuals, with the following results:

1. The respiratory pause was found to be usually less than 2·5 seconds for a single act of deglutition, and in no case did it exceed 3·5 seconds, while ·5 second was the shortest recorded.

2. During the swallowing of liquid (water) the pause was appreciably shorter than when solid food was taken, in the majority of subjects.

3. The normal respiratory rate of the individual did not appear to influence the length of the apnœic period.

4. A strikingly larger percentage of acts of deglutition occurred during the expiratory phase of respiration; this was particularly noticeable when solid food was swallowed, as seen in the following table:

	Apnœa during		Average duration
	Inspiration	Expiration	
A. Solid food	13 (11·5 %)	100 (88·5 %)	1·5 secs.
B. Liquid	21 (29·0 %)	51 (71·0 %)	1·4 secs.

5. The respiratory movement was resumed, after the apnœic pause, at the point at which it had been arrested. There were a few exceptions, the majority of which showed an interrupted inspiration followed by expiration. In some cases an expiratory act followed the pause when this occurred at what appeared to be the limit of normal expiration.

An improved form of Barfoed's Reagent. By H. E. Roaf.

In another communication (1) it was pointed out that the reduction of cupric salts is dependent on the concentration of hydroxyl ions. Thus maltose and lactose cannot reduce an acid solution of cupric salts, but the stronger reducing monosaccharides can reduce cupric acetate.

As Barfoed's reagent requires to be boiled for from three to five minutes before reduction occurs it is desirable to use a reagent which will show reduction in a shorter time. By reducing the acidity it is possible to increase the ease of reduction and at the same time not make the reagent so sensitive as to become reduced by maltose or lactose.

Several modifications were tried such as using copper butyrate but the most satisfactory solution was made by adding sodium acetate to copper acetate in acetic acid. In this way the ionisation of the acetic acid is decreased and the reagent is reduced by glucose on merely bringing the mixture to the boil. By decreasing the acidity hydrolysis of disaccharides is decreased but the reagent will show reduction with less of the hydrolysed product so prolonged heating should be avoided. If the acidity is too far decreased heating will cause hydrolysis of the weak salt, thus cupric oxide will be precipitated.

In order to show the possible variations in the reagent the following experiments are quoted.

Sol.	Copper acetate g.	Glacial acetic acid c.c.	Water to c.c.	
A	10	0·0	100	not all dissolved
B	10	0·2	100	„ „
C	10	0·5	100	„ „
D	10	1·0	100	„ „

	Sol. A c.c.	16·7 % sod. acetate c.c.	Water c.c.		Sol. B c.c.	16·7 % sod. acetate c.c.	Water c.c.
1	10	5	0	1	10	5	0
2	10	3	2	2	10	3	2
3	10	1	4	3	10	1	4
4	10	0	5	4	10	0	5
	Sol. C				Sol. D		
1	10	5	0	1	10	5	0
2	10	3	2	2	10	3	2
3	10	1	4	3	10	1	4
4	10	0	5	4	10	0	5

	Solution c.c.	1 % glucose c.c.	Duration of boiling		
			Immediately	5 mins.	10 mins.
A 1	5	5	+	+ + +	+ + +
A 2	„	„	+	+ + +	+ + +
A 3	„	„	+	+ + +	+ + +
A 4	„	„	+	+ + +	+ + +
B 1	„	„	+	+ + +	+ + +
B 2	„	„	+	+ + +	+ + +
B 3	„	„	+ +	+ + +	+ + +
B 4	„	„	+ +	+ + +	+ + +
C 1	„	„	+	+ +	+ +
C 2	„	„	+	+ +	+ +
C 3	„	„	+	+ +	+ +
C 4	„	„	0	+	+ +
D 1	„	„	0	+	+ +
D 2	„	„	0	+	+ +
D 3	„	„	0	+	+ +
D 4	„	„	0	+	+

No reduction = 0.
Amount of reduction shown by number of +'s.

1 p.c. maltose and 1 p.c. lactose did not show reduction in less than five minutes and at the end of that time only in a few of the mixtures but a black precipitate came down in many of the mixtures after boiling for five minutes.

As the result of a number of such experiments the most satisfactory reagent was found to be

copper acetate 50 g.,
sodium acetate 50 g.,
glacial acetic acid 5 c.c.,
water to 1000 c.c.

With this reagent a reduction is obtained with a 0·1 p.c. glucose solution on merely bringing to the boil whilst pure specimens of 1 p.c. maltose and 1 p.c. lactose do not show reduction under the same conditions.

It may be possible to increase the sensitiveness of the reagent so as to be able to make a quantitative estimation of monosaccharides in the presence of disaccharides but in any case this reagent is an improvement on Barfoed's reagent for the purpose of showing hydrolysis of maltose and lactose by enzymes(2).

(1) Roaf. Proc. Physiol. Soc. Nov. 1920.
(2) H. E. Roaf. Biochem. Journ. 3. 182. 1908.

The physiological cost of colliers' work. By A. D. WALLER and G. DE DECKER.

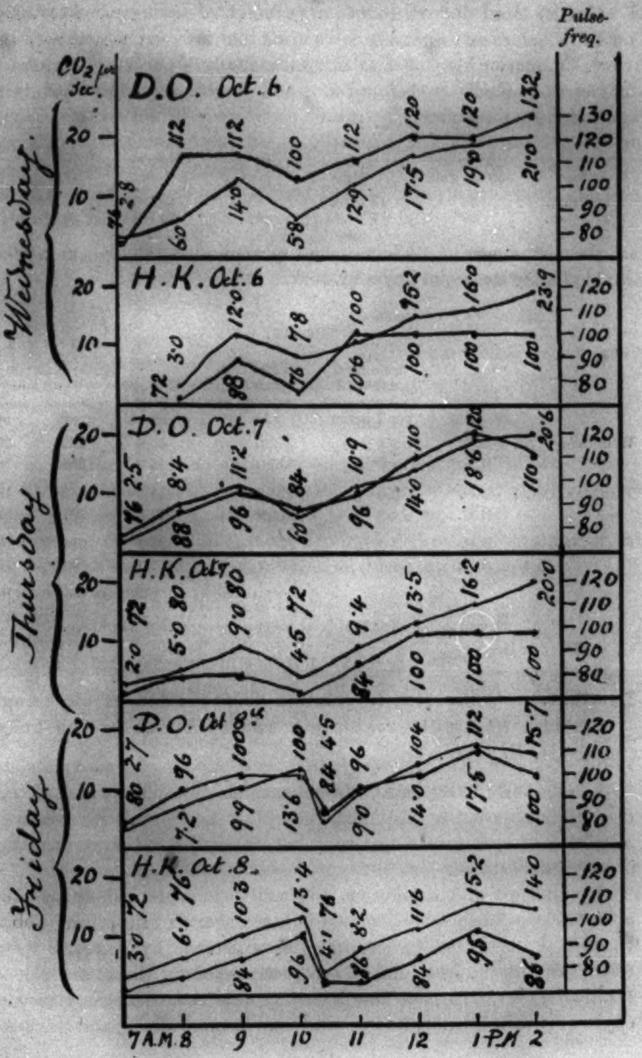
Our observations were taken hourly during the morning shift of seven hours from 7 a.m. to 2 p.m. during three successive days upon two colliers at the Cwm Colliery near Pontypridd by courtesy of the Great Western Colliery Co., and thanks to the excellent arrangements made for our comfort by Mr Rees, manager of the mine.

The procedure was to collect expired air for 30 secs. each hour from each of the two colliers at the coal face, with least possible interruption of their work which consisted in "getting coal" and loading it on trucks. The conditions of work were favourable, the seam being 4 ft 6 in. to 5 ft thick, the mine dry and the temperature not above 20° C.; the depth was 2000 feet and the barometer averaged 31 in. Hg; ventilation good.

The coal face is about 400 yards from the mine shaft, the volume of expired air and its percentage of CO_2 were measured at once in the mine stable in a vacant stall prepared for us by Mr Rees, and no difficulty occurred in the hourly collection from the two subjects, who lent themselves to the proceedings with the utmost good-will; G. De Decker found it possible during each half minute of collection, to take a pulse count, the work was practically maximal and constant except towards the end of the seventh hour, the wage being dependent upon the tonnage got. For the three days of our observation this was 4·5, 4·0, and 3·5 tons per collier at this particular face, at which three colliers were at work. The unit of delivery being the truck of 1·5 tons caused the rate of work to vary slightly at the end of the shift according as the men were filling their last truck under pressure, or had filled it just before time was up, *e.g.* on Friday when the last truck was despatched at 1.50 p.m. The observations are summarised in the following graphs of CO_2 in c.c.'s per sec. and of pulse-frequency in beats per minute.

There is a general parallelism in the rising and falling ordinates representing CO_2 discharge and pulse-frequency. Both curves rise throughout the shift, with a fall at 10 a.m. corresponding with the luncheon interval. On the third day (Friday) the men had finished work at 1.55 and were beginning to cool down.

The most remarkable feature in the curve formed by the CO_2 ordinates during the seven hours' shift is its rise from start to finish (except as just stated). The work is practically continuous and constant, so that a rise from *e.g.* 6 c.c. per sec. at the end of the first hour to 19 at the end of the sixth hour is most probably significant of increasing physiological cost of work, *i.e.* decreasing mechanical efficiency.


CO2 per sec.
Pulse-freq.
Wednesday
Thursday
Friday
D.O. Oct. 6
H.K. Oct. 6
D.O. Oct. 7
H.K. Oct. 7
D.O. Oct. 8
H.K. Oct. 8
20
10
130
120
110
100
90
80
7 A.M. 8
9
10
11
12
1 P.M.
2

On the third day we found opportunity of taking an observation on D. O. before and after his day's work that is in agreement with this view. Comparing his cost of walking horizontally 60 paces (approximately 50 metres) immediately before 7 a.m. and immediately after 2 p.m. with the following result:

Time	Time of 50 m. walk	Volume	CO_2 per cent.	CO_2 c.c. per sec.	$\frac{CO_2}{KgM}$ (hor.)
6.45 a.m.	32″	13 litres	3·2	13·0	0·105 c.c.
2.15 p.m.	30″	17 „	4·5	25·5	0·221 „

i.e. the cost of moving 1 kilogramme 1 metre horizontally has more than doubled after the seven hours of work.

SUMMARY.

		Estimated output of coal per man per 7 hours	Total cost gross CO_2 exhaled in 7 hours	CO_2 per hour (gross)	Kalories per hour	
					gross	net
Wednesday, Oct. 6	D.O.	4·5 tons	313·5 litres	44·8	262	202
	H.K.*	4·5 „	315·0 „	45·0	264	198
Thursday, Oct. 7	D.O.	4·0 „	290·3 „	41·5	243	183
	H.K.	4·0 „	246·9 „	35·3	207	141
Friday, Oct. 8	D.O.	3·5 „	256·6 „	36·7	216	156
	H.K.	3·5 „	230·4 „	32·9	193	127

average net cost = 168

* Allowance made for the first hour when no observation was taken.

Le Chatelier's theorem in relation to reduction of oxyhæmoglobin, neutrality regulation and oxidation in the body.

By H. E. ROAF.

Le Chatelier stated that a reaction which takes place with a change of state is impeded by that alteration. This law is well recognised in relation to temperature and pressure but its relation to change of acidity is not usually stated.

By analogy with sulphurous and sulphuric acids and nitrous and nitric acids, oxyhæmoglobin should be a stronger acid than hæmoglobin. In 1913 I attempted to measure this difference by mercuric oxide electrodes but the hæmoglobin was precipitated by the mercury ions. Haldane, Christiansen and Douglas obtained results in favour of oxyhæmoglobin being a stronger acid than hæmoglobin (1), and Parsons has obtained results which can be interpreted in the same sense(2). If oxyhæmoglobin is a stronger acid than hæmoglobin acid should inhibit the formation of oxyhæmoglobin, which is in fact the case.

I have endeavoured to obtain experimental justification of this application of Le Chatelier's theorem. Oxidation of sulphurous to sulphuric acid should occur less easily in an acid solution and sulphurous acid is known to be more stable than sulphites. Unfortunately addition of alkali to sulphite also inhibited the oxidation to sulphate but this inhibition may be due to some complicating factor.

1·3 p.c. solution of sodium sulphite 5 c.c. = 50·4 c.c. iodine solution.

Na_2SO_3 solution	After 4 hours at 42° C. require c.c. iodine	
10 c.c. + 10 c.c. *N*/10 HCl	82·7	All diluted and acid added so as to contain the same concentration of acid during titration
10 c.c. + 1 c.c. *N*/10 HCl + 9 c.c. water ...	16·1	
10 c.c. + 10 c.c. water	0·2	
10 c.c. + 1 c.c. *N*/10 NaOH + 9 c.c. water	22·0	
10 c.c. + 10 c.c. *N*/10 NaOH	36·5	

A number of experiments gave similar results.

Oxidation of nitrites was not tested because nitrous acid is less stable than nitrites. In this case nitrous acid does not form an equivalent quantity of nitric acid so that the acidity is decreased and not increased as would be the case if it were all oxidised to nitric acid, *e.g.*

$$3HNO_2 = HNO_3 + 2NO + H_2O.$$

This equation supports the thesis than an increase in acidity aids a reaction which takes place with a decrease of acidity.

Turning to the oxidation and reduction of metallic salts one finds that the results are what would be expected if Le Chatelier's theorem is to hold. For example cupric salts being divalent and stronger bases than cuprous they should be more easily reduced in an alkaline solution. We find that all reducing sugars will reduce an alkaline solution of cupric salt but only the stronger reducing monosaccharides can reduce an acid solution of cupric acetate.

Other metallic salts are also more easily reduced in alkaline than in acid solutions.

In addition to their relation to the reduction of hæmoglobin these observations have many applications in physiology.

Conversion of ammonia into urea is accompanied by a decrease in alkalinity therefore increase in acidity will inhibit the formation of urea and increase in alkalinity will increase the conversion into urea so that ammonium salts will disappear from the urine. These are well known occurrences.

Conversion of methyl glyoxal to lactic acid is accompanied by an increase in acidity, thus Dakin and Dudley had to add something to neutralise the lactic if they wished to obtain a good yield of the acid(3).

Oxidation in the tissues is dependent on the reaction being maintained near the neutral point. If an increase in acidity occurs at one stage of oxidation that stage will be inhibited by acid and the removal of the acid product will be inhibited by alkali. The converse holds if an alkaline product is formed during oxidation. It may be that increased acidity or alkalinity may exert their influence on different constituents but one sees a possible explanation of the narrow range of hydrogen ion concentration at which life is possible.

The influence of decreased oxygen tension on the respiratory centre can be brought into relation with the carbon dioxide tension in this way. With a decrease in the oxygen tension less oxyhæmoglobin is formed and less carbon dioxide will be removed from the blood for the same tension of carbon dioxide than if the blood were fully oxygenated. This less oxygenated blood, which has a diminished capacity for combining with carbon dioxide, on reaching the respiratory centre will not remove so much carbon dioxide from the centre. Therefore lack of oxygen is equivalent to the addition of acid to the blood thus accounting for the apparent lowering of the threshold of the respiratory centre.

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The passage of urea through the cells of the tubules of the kidney of the frog. By J. A. Menzies.

According to Cushny's view of the formation of urine, the cells of the tubules absorb water and certain solids, but do not secrete any of the urinary constituents(1). Experiments have been carried out to ascertain whether urea passes from the intertubular vessels into the tubules of the kidney of the frog.

It has previously been shown(2) that urine is formed in the frog when the glomeruli are perfused with Ringer's fluid under normal pressure, and that, when the intertubular vessels only are similarly perfused under normal pressure, no urine is formed. In the present experiments the method used by Bainbridge, Collins and Menzies was adopted. The aorta was perfused with oxygenated Ringer's solution under a pressure of about 24 cm. of water. The renal portal veins were simultaneously perfused with Ringer's solution containing 0·02 p.c. of urea under a pressure of about 12 cm. of water. In these circumstances the urine invariably contained urea. When both glomeruli and intertubular vessels were perfused with Ringer's fluid alone, the urine formed contained no urea.

The kidneys perfused in this way show considerable œdema. The thin wall of the intertubular vessel, which is normally closely applied to the epithelial cells of the tubule, is separated from the cells by a wide space, but there is no breach in the continuity of the layer of tubule-cells.

In order to increase the osmotic pressure of the perfusing fluids, 2 p.c. of gum(3) was added to each in all the later experiments. The result of this addition was that there was no appreciable œdema in the perfused kidneys, while urea passed into the urine as usual.

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Stereoscopic effects produced by pictures of different sizes.

By E. H. Hankin and H. Hartridge.

If, after taking a photograph, the camera is moved a few inches nearer the object and another photograph is taken, then the two pictures, when placed side by side in a stereoscope, show a restricted portion of the resulting picture in stereoscopic relief.

The relief appears on the right side of the picture if the right hand picture is the smaller of the two. The opposite side of the picture shows a pseudostereoscopic appearance. No relief effect is produced along the central vertical line of the picture, presuming that the point towards which the camera was moved was situated on this line.

If the pictures are each divided into two equal pieces by a vertical cut, and if two of the half pictures are interchanged, then if the halves of the larger picture are centrally placed stereoscopic relief is seen over the whole of the combined picture, except on the central line. If the halves of the larger picture are placed in the alternative position then pseudostereoscopic effect is produced over the whole picture, again excepting the central line. When the halves are arranged to produce stereoscopic relief it may be noticed that the effect is greatest peripherally and decreases progressively towards the central line.

If the approach of the camera between taking the two photographs is 12 inches, then exaggerated stereoscopic relief, as seen in ordinary commercial stereoscopic views, is obtained. If the approach is only three inches, what may be described as normal stereoscopic relief is produced, though the difference in size of the resulting pictures is too small to be measured without a micrometer-microscope.

The explanation of the phenomenon appears to be as follows. Referring to Diagram A, (1) and (2) represent the nodal points of the right and left eye. *X* and *Y* are two points in space to the right of the observer situated on a line which when prolonged meets a point situated between the two eyes. To his right eye *Y* appears to the *left* of *X*, while to his left eye *Y* appears to the *right* of *X*.

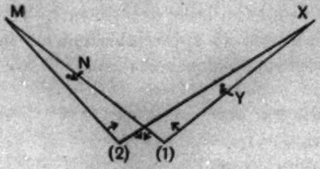

Diagram A.

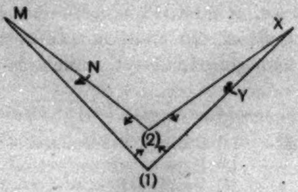

Diagram B.

In Diagram B, (1) and (2) represent the first and second positions of a camera lens when used for taking photographs of different sizes. On the first picture (taken from position (1)), *Y* appears to the *left* of *X*, whereas on the second picture (taken from (2)), *Y* appears to the *right* of *X*.

If then the right hand halves of the photographs are presented to the right and left eyes, so that the right eye has the first photo, and the left eye has the second, *X* and *Y* will hold their correct relative positions and will, therefore, be seen stereoscopically. Hence, stereoscopic relief is found on the right-hand side of the picture.

Referring once more to Diagram A, *M* and *N* are two points in space to the left of the observer and situated on a line which when prolonged meets the central point between the two eyes. To his right eye *N* is to the *left* of *M*, whereas to his left eye *N* is to the *right* of *M*.

In Diagram B, on photo (1), *N* appears to the *right* of *M*, whereas on photo (2), *N* appears to the *left* of *M*. If then, as before, the photos are combined with the help of a stereoscope, so that the right eye has photo (1) and the left eye photo (2), it may be seen that the photos give precisely the reverse effect to that observed visually, for whereas to the right eye *N* is to the *left* of *M*, on the right photo *N* is to the *right* of *M*, and *vice versa*. Vision of these halves of the pictures must therefore be pseudostereoscopic.

But if now the photos are cut in halves and the right-hand halves arranged as before (photo (1) opposite the right eye and photo (2) opposite the left), while the other half pictures are transposed so that photo (1) is opposite the left eye and photo (2) opposite the right eye, then the position of *M* and *N* will be the same as they are in ordinary vision and therefore when combined they must be seen in stereoscopic relief. That it is not the difference in scale of the two photos which produces the relief can be shown by experiment as follows: If two precisely similar photos be combined stereoscopically no relief is of course observed, if now a weak convex lens be placed before one of the lenses of the stereoscope, so as to magnify the image to this eye more than the other and thus alter the scale of the two pictures no trace of relief can be seen.

Changes in the adrenal bodies and the thyroid resulting from inanition. By SWALE VINCENT and M. S. HOLLENBERG.

Our previous investigations on "The Effects of Inanition upon the Adrenal Bodies and other Organs (1)," leads us to conclude that during the early stages of inanition there is an increase in the amount of adrenin in the adrenal bodies, and that during the latter stages the amount of adrenin is greatly reduced.

The above results were arrived at as follows. An adrenal from a normal dog was taken and one from a starved dog. Both were weighed and an extract of each made. Saline solution was then added to each in proportion to its weight. The relative strengths of the two extracts were then compared by intravenous injection of equal quantities into a dog, the corresponding rise of blood pressure indicating the relative strengths of the adrenin content in the gland.

We have now carried our investigation further and determined chemically the amount of adrenin contained in the adrenals of normal and inanition animals, utilising the method devised by **Folin, Cannon** and **Denis**(2).

Our method of procedure was as follows. The weighed glands were ground up in a mortar with 1·5 c.c. of 0·1 *N* HCl and rinsed into a conical flask with 4·5 c.c. of water. The acid mixture was then heated to boiling, 5 c.c. of a 10 p.c. solution of sodium acetate was added, and the mixture again heated to boiling to precipitate the proteins. The mixture was then filtered and the filtrate collected into a 100 c.c. measuring flask. At the same time 1 c.c. of a standard uric acid solution was pipetted into another 100 c.c. flask. To each flask 2 c.c. of uric acid reagent was added and 20 c.c. of saturated sodium carbonate solution. After standing for two or three minutes the solutions were diluted to the 100 c.c. mark, shaken and the colour comparison made with a Kober colorimeter with the uric acid standard at 20 mm.

In our investigation we utilized 17 rats in all. Six were used as controls. Five were killed after 2–3 days starvation. Six were killed after 10–13 days starvation.

The following tables give the average percentage of the body weight of the organs of the rats and the adrenin content of the adrenals.

The results confirm those of our previous investigation namely that during the early stages of inanition there is an increase in the amount of adrenin in the adrenal bodies, and that during the later stages the amount of adrenin is greatly reduced.

To ascertain whether the effects of inanition upon the adrenals were permanent or temporary we starved two rats for a period of 12 days and then restored them to their normal weight. We found that there was no hypertrophy and that the adrenin content of the glands was normal.

Throughout our investigations we have noticed that the thyroids as well as the adrenals hypertrophy during inanition. This result has been obtained so constantly in this investigation that we sectioned the thyroids of these inanition rats. We found that the colloid substance which is normally contained in the vesicles was almost entirely lacking. The significance of the hypertrophy and the disappearance of the vesicular colloid is as yet not clear to us.

Herring(3) found that the average content of 13 male rats' adrenals was 0·224 p.c. adrenin. The figure for adults was ·25 p.c. His rats were of various ages. **Kuriyama**(4) used five males and six females which were nearly all adult. His percentage of adrenin content was 0·26 p.c. Both the above results were obtained by Folin's method. The average percentage of adrenin content of our controls is 0·27 p.c.

TABLE I. Comparison of Weights of Organs of Normal and Inanition White Rats.

	Normal animals		Inanition animals (2–3) days		Inanition animals (10–12) days	
	Males (3)	Females (3)	Males (2)	Females (3)	Males (4)	Females (2)
Average total weight	207 gm.	176·5 gm.	212 gm. 189 gm.	210 gm. 192 gm.	264 gm. 172 gm.	247 gm. 164 gm.
Brain	0·925 %	0·887 %	0·916 %	0·875 %	0·968 %	0·900 %
Lungs	0·596	0·612	0·555	0·588	0·602	0·687
Heart	0·474	0·502	0·470	0·491	0·556	0·598
Spleen	0·199	0·235	0·189	0·201	0·170	0·189
Adrenals	0·01670	0·0170	0·0188	0·0190	0·0565	0·060
Thyroids	0·0152	0·0153	0·0160	0·0172	0·420	0·423
Liver	5·62	5·76	5·00	4·947	3·071	3·10
Testes	1·253	—	1·223	—	1·022	—
Ovaries	—	0·027	—	0·025	—	0·023
Kidneys	0·830	0·845	0·822	0·832	0·829	0·833
Submaxillary glands	0·342	0·322	0·324	0·309	0·260	0·267

TABLE II. Adrenin Content and Adrenal Weight compared with Body Weight, in Normal and Inanition Rats.

No.	Period of starvation	Weight before starvation	Weight when killed	Weight of adrenals	Adrenin content of adrenals
1	—	—	200·0 gm.	0·0320 gm.	0·093 mg.
2	—	—	209·5	0·0365	0·086
3	—	—	213·5	0·0346	0·094
4	—	—	169·0	0·0304	0·092
5	—	—	185·0	0·0296	0·088
6	—	—	175·5	0·0297	0·094 mg., mean 0·094 mg.
7	69 hours	201·5 gm.	180·0	0·0342	0·132
8	70	222·5	198·0	0·0368	0·141
9	67	186·0	178·5	0·0339	0·134
10	72	242·0	230·5	0·0432	0·143
11	65	202·0	187·0	0·0344	0·128 mg., mean 0·135 mg.
12	10 days	241·0	165·0	0·0924	0·035
13	11	280·0	192·5	0·1094	0·030
14	10	265·0	176·0	0·0993	0·027
15	10	270·0	154·5	0·0870	0·033
16	12	268·5	176·5	0·1056	0·040
17	12	226·0	151·5	0·0899	0·031 mg., mean 0·032 mg.

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A simplified blood gas volumeter for the use of students[1].

By D. T. Harris.

This was designed to enable students to follow the events in the Ferricyanide method of Haldane and to serve as a preliminary exercise before proceeding to the excellent Blood gas Manometer of Barcroft which involves so many mechanical difficulties for the student. Its use can be best ascertained from the full working instructions given to the student:

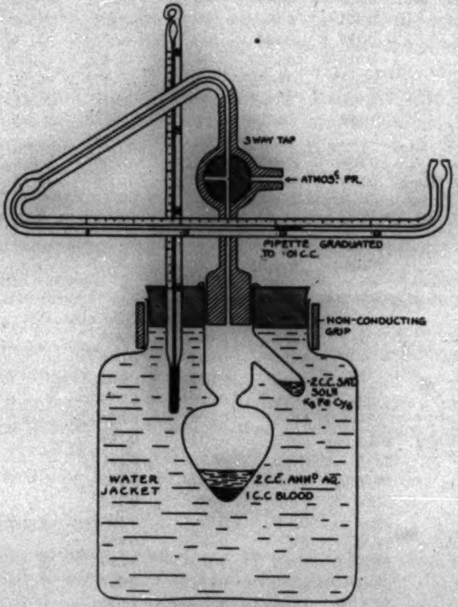
3 WAY TAP
ATMOS^c PR.
PIPETTE GRADUATED TO ·01 C.C.
NON-CONDUCTING GRIP
·2 C.C. SAT SOL^N K_3FeCy_6
WATER JACKET
2 C.C. AMM^O AQ.
1 C.C BLOOD

Fig. 1. (Scale = ½)

DETERMINATION OF THE OXYGEN CONTENT OF VENOUS BLOOD.

The venous blood provided has been collected from a vein into a tube containing a little powdered potassium oxalate and has been kept under liquid paraffin.

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[1] This apparatus is made by Messrs Baird and Tatlock.

i. DETERMINE THE VOLUME OF O_2 NECESSARY TO SATURATE 1 C.C. VENOUS BLOOD.

1. Into the bulb introduce 2 c.c. (approx.) of dilute ammonia ($\frac{1}{2}$ p.c.) containing a little saponin (as much as would be taken up by the terminal mm. of a penknife).

2. By means of a 1 c.c. standard pipette fitted with a rubber teat, introduce 1 c.c. of the venous blood under the surface of the dilute ammonia in the bulb, without agitating.

3. Into the side-tube put about 0·2 c.c. of a freshly made saturated solution of potassium ferricyanide. (Fig. 3.)

4. With the 3-way tap open and the mercury thread near the middle of the graduated pipette, fix the stopper firmly in the bulb neck with the graduated pipette in the same plane as the side tube. (Fig. 4.)

Adjust the cork of the water-jacket so as to make the pipette horizontal.

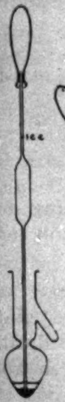

Fig. 2.

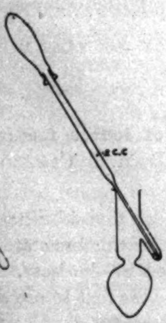

Fig. 3.

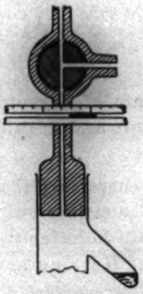

Fig. 4.

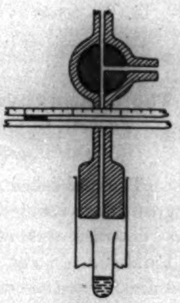

Fig. 5.

5. Note the temperature, wait 5–10 minutes for the bulb to take the temperature of the water which should be at room temperature.

Rotate the 3-way tap through 180° leaving the bulb in communication with the graduated pipette only. (Fig. 1.) Read the temperature and the position of the mercury thread every minute until constant[1].

6. Rock the bottle in a plane at right angles to the mercury thread and side-tube (to avoid spilling), thus thoroughly mixing the blood with the ammoniated water and spreading the laked blood in a thin film over the walls of the pear-shaped bulb. (To avoid heating the water-jacket, grip its neck high up or surround it with a rubber band cut from the inner tube of a cycle tyre.)

[1] If the Hg thread has a tendency to stick either tap the water-jacket gently on the table or have the pipette previously rinsed with acidulated water (5 p.c. H_2SO_4).

7. Read the temperature (which should remain unaltered) and the position of the mercury thread.

Since the pipette is graduated in $\frac{1}{10}$ths c.c. and 1 c.c. of blood has been used, each small division on the graduated pipette corresponds to 1 p.c. of the volume of blood. Hence, the p.c. O_2 necessary to saturate venous blood (at this temperature) is given directly by the excursion of the mercury, which is due to the shrinkage in the volume of the air in the bottle.

ii. DETERMINATION OF THE OXYGEN CAPACITY OF THE SAME BLOOD.

8. Open the 3-way tap to the atmosphere, move the mercury thread to the left, rotate the stopper through 90° so that the pipette is now at right angles to the side-tube. (Fig. 5.) Repeat operation 5.

9. Tilt the whole apparatus until the potassium ferricyanide solution runs into the bulb. Repeat operations 6 and 7.

The net O_2 given off by the blood (at this temperature) is then given by the excursion of the mercury. [This figure is termed the O_2-capacity.]

Calculation.

O_2 content of the venous blood = O_2-capacity − O_2 necessary to saturate the venous blood.

iii. DETERMINATION OF THE CO_2 CONTENT OF THE SAME BLOOD.

Using tartaric (or lactic) acid in operation 3, repeat 3–7

The comparative effects of thyroid and of iodide feeding on growth in white rats and in rabbits. By A. T. CAMERON and J. CARMICHAEL.

The published data concerning the effect of thyroid extract on growth are conflicting. The majority of the earlier workers stated that a decrease was obtained, Schafer(1) obtained an increase, E. R. Hoskins(2), no effect, and Herring(3), who was the first to use control animals from the same litter, obtained a slight decrease.

None of these observers used an accurate dosage, based on the (changing) body-weight of the animal, and this is almost certainly the chief cause of the varying results, since the thyroid effect is proportional to the thyroid fed.

Iscovesco(4), feeding a thyroid "lipoid" preparation (Fenger's results(5) throw doubt on the lipoid nature of this preparation) obtained marked hypertrophy in many of the organs of the rabbit (and an increase in rate of growth). Hoskins feeding both desiccated and fresh thyroid gland, obtained the same hypertrophy with the young white rat, the degree of hypertrophy being proportional to the size of dose. Herring confirmed these results.

During last winter we carried out a number of experiments in which experiment- and control-animal were of the same sex, and from the same litter, and the thyroid (and sodium iodide) dose given daily was based on the day's weight of the rat fed. Thyroid was fed in proportions of 1: 20000, 1: 10000, 1: 5000, and 1: 2000 of desiccated gland to body-weight. Three preparations were used, a Merck prepaxation at least nine years old, containing 0·39 p.c. iodine, a hog-thyroid preparation containing 0·34 p.c., and a sheep-thyroid preparation containing 0·18 p.c. iodine; the two latter were prepared in December 1919, and were obtained from the Armour laboratories through the kindness of Dr F. Fenger. In all 40 male and 17 female rats were used. Of these 31 male and 16 female were from nine litters; ten controlled experiments were carried out with thyroid, and four with sodium iodide. The initial age of feeding these varied from 40 to 60 days. The remaining ten animals were fed large doses of thyroid in an endeavour to induce tetany. Unlimited bread and milk was given as diet. The following results were obtained:

(1) Continued small doses of desiccated thyroid gland fed to young white rats produce (*a*) a definite and invariable decrease in the rate of growth, (*b*) hypertrophy of the organs concerned with increased metabolism—heart, liver, kidneys, adrenals, etc. (confirmatory of Hoskins and Herring), (*c*) disappearance of fat (confirmatory of Hoskins and Herring).

(2) The decrease in rate of growth is proportional to (*a*) the amount of thyroid fed, (*b*) the iodine content of the thyroid fed.

(3) There is some evidence that long continued small dosage, at any rate with female rats, ceases to produce this effect, but this is probably due to the fact that the hypertrophy above-mentioned balances the fat-loss.

(4) The hypertrophy produced varies with size and length of application of dose, and appears to be proportional to the iodine content of the thyroid fed.

(5) Sodium iodide, fed in quantities varying from amounts equal in iodine content to the thyroid doses to amounts one hundred times as great, produces no effect on the rate of growth, and no hypertrophy.

(6) The thyroid effect is not due to toxicity from high protein content or from compounds resulting from autolysis occurring during preparation of the thyroid. Desiccated liver, given as a control, produced negative effects.

(7) Both thyroid and iodine feeding increase the colloid in the thyroid (confirmatory of Fordyce(6) and Kojima(7)). No histological changes were observed in the hypertrophied tissue.

(8) Two cases of tetany were observed, the first accidentally, and both with young rats: onset of tetany occurred between the 50th and 60th day after feeding a fairly heavy dose for a fortnight. Older rats are less susceptible to the action of thyroid.

(9) Desiccated thyroid gland does not appear to deteriorate with age.

(10) The effects of vitamine deficiency appear to be increased when thyroid is fed concurrently.

One litter of rabbits (three male and two female) was tested; the two male and one female animals fed thyroid all showed decrease in growth and hypertrophy of organs as compared with the control animals.

The essential difference between the result of thyroid and of iodide feeding to young white rats suggests that decrease in rate of growth and hypertrophy of such organs as the heart, liver, and kidney can be used as discriminatory tests for preparations alleged to be the essential thyroid secretion.

The proportionality of the action to iodine content, in harmony with other observations on the physiological and metabolic action of thyroid extract, is to be expected if thyroxin is the essential constituent of the thyroid, since, according to Kendall(8), this occurs combined in the gland in amount invariably corresponding to one-fourth of the total iodine content.

The full details of this work will be published at an early date.

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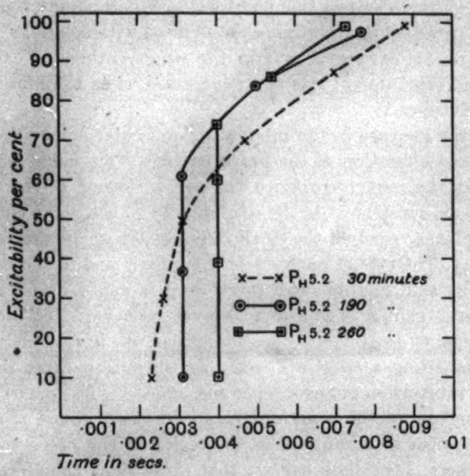
The conductivity of the nerve ending in acid solutions. By YNGVE ZOTTERMAN.

The following experiments were carried out to see what differences existed in the reaction of the nerve ending and the nerve to alteration in the p_H of the perfusing fluid. Gastrocnemius sciatic preparations were set up in a Lucas' perfusion chamber, the nerve being surrounded with ordinary Ringer p_H 6·5 and the muscle perfused with solutions of different p_H. These were made up in accordance with the formulæ of Mines and the p_H was measured by indicators. In the more acid solutions the muscle itself is liable to damage: thus in a p_H of 4·5 the muscle ceased to contract after a time of about five hours; in p_H 4 after four hours and in p_H 3 in less than half-an-hour (16–17° C. summer frogs). To test the effect on the nerve endings the recovery curve of the nerve was determined using the summated contraction of the muscle as an index of the success of the second stimulus. When the whole preparation was in p_H 7 no change in the curve could be detected in 24 hours. In p_H 5·2 there was as the figure shows an increase in the least internal for muscular summation from ·0023 sec. to ·0031 sec. after the muscle had been perfused for three hours. After four hours the interval increased to ·004 sec. The shift in the upper part of the curve probably depends on the action on the nerve of the ordinary Ringer's fluid which was slightly on the acid side of neutrality.



We have found an increase in the least interval for muscular summation in about ten preparations in different acid solutions ranging from p_H 6·5 to p_H 4·5. The increase comes on more rapidly in the more acid solutions, but in p_H 4·5, the strongest acid solution we could use without damaging the muscle, we never found an increase in the least interval for muscular summation in less than two hours.

Before we can attribute this increase in the interval to an alteration in the nerve endings two other possibilities must be considered. In the first place it might be due to an increase in the absolute refractory period of that part of the nerve which is exposed to the acid solution. If so, we should expect to find the same alteration in the refractory period if the nerve were perfused with acid solutions and the nerve endings and the muscle left in neutral Ringer, but under these conditions we have found no change in the refractory period although the nerve has been exposed to a fluid of p_H 5 for more than six hours. The other possibility is that the absolute refractory period of the muscle might be increased by the acid fluid. To test this point we have determined the refractory period in curarised sartorius muscles perfused with solutions of different p_H. The refractory period was in the neighbourhood of ·006 sec. at 15° C., and this interval was never increased when the muscle was left in acid solutions.

Thus the increase in the interval for muscular summation must be due to some alteration of the nerve endings. This might be either an increase in the refractory period through a slowing of the rate of recovery or an impairment in the conductivity of the nerve ending which would no longer conduct the small impulses set up in the early stages of recovery. The rate of recovery of the muscle and of the nerve is not retarded by acid solutions and therefore it is unlikely that the change in the nerve ending is due to a delayed recovery. This argument is strengthened by the following considerations. We have sometimes found when stimulating a muscle directly an extremely short interval for muscular summation of about ·0004 sec. at 15° C. Bazett(1) sometimes found intervals of the same order and Lucas(2) found a very short chronaxie when he stimulated in the region of the nerve endings, but not elsewhere. They both suggest that these short time-relations refer to the nerve endings. Now we have never found any increase in this interval after perfusion of several hours with acid solutions and it is therefore extremely probable that the refractory period of the nerve ending is not increased by acid.

We may conclude that perfusion with fluids on the acid side of neutrality causes the nerve ending to conduct with a decrement. The effect can be detected with a fluid of p_H 6·5 but it does not appear for several hours even in the more acid solutions. As the nerve itself shows no alteration in conductivity after six hours' perfusion with a fluid of

p_H 5 it seems likely that the nerve ending is more sensitive than the nerve fibre to an increased H ion concentration in the perfusing fluid.

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Dispersivity and surface phenomena. By William Alexander Osborne.

Finely divided matter when it attains to the degree of dispersivity possessed by colloidal solutions displays maximal optical activity and maximal catalytic power. That there should be for a dispersed phase a critical diameter with respect to optical qualities is explained by the relation to the wave lengths of light in the visible spectrum. But to my knowledge no explanation has been forwarded for the diminution of catalytic action when the dispersivity exceeds the ordinary colloidal and approaches that of true solutions.

We will assume catalysis to be causally associated with specific surface and the special qualities which a surface displays. Now the phenomena of surface are referable to unbalanced molecular forces. Each molecule at the surface is under a strain caused by the pull of all those other molecules which lie within the range of molecular attraction and this range we may take roughly to be something like ten times the molecular diameter. If now the diameter of a colloid particle is smaller than the range of molecular attraction the strain must be less owing to the diminished number of molecules whose forces come into play. Finally when the particle is of molecular size lack of balance may be said to be absent.

Another way of approaching the problem is to regard only the surface of the dispersion medium in contact with the surface of the dispersed phase. Here we have the site of adsorption phenomena. Now when the colloid particle approaches molecular size the attraction of the molecules of the dispersion medium may be able to act further than the diameter of the particle and hence the lack of balance in the surface of the medium in contact with the dispersed phase is diminished. The simplest instance of this is to be found in a bubble within a fluid. The excess pressure, *i.e.* the pressure over and above atmospheric and hydrostatic in a bubble

$$=p=\frac{2T}{R},$$

where T is the surface tension of the liquid, and R the radius of the bubble. That is to say the pressure in the bubble has a simple inverse linear relationship to the radius. If the radius approaches the infinitely small will the pressure, as stated in some books on physical chemistry, approach the infinitely great? It will not for this reason that when the diameter of the bubble is less than the range of attraction of the molecules of the liquid, such molecular attraction will play *through* the bubble and the molecules that bound the bubble will no longer be acted upon in an unbalanced manner. Consequently when the bubble is of molecular size the surface tension and the excess pressure become zero.

It appears therefore that a quantitative estimation of catalytic power with varying diameter of colloidal dispersed phase would give an indirect method of measuring the range of molecular attraction.

The influence of coagulation upon the reaction of blood. By J. P. Ross.

Among the many sources of difficulty attending the use of the hydrogen electrode for determination of blood reaction is that associated with an accumulation of fibrin on the surface of the platinum electrode. According to Michaelis(1) this causes an apparent acidification of the blood; though experiments made by Höber(2), also with the hydrogen electrode, with the object of determining the influence of coagulation on blood reaction, showed no appreciable difference. In view of the difficulty of obtaining satisfactory results by the use of the hydrogen electrode, advantage was taken of the method recently described by Dale and Evans(3) to investigate this point, since the method enables comparative results to be obtained of the same order of accuracy as those yielded by the hydrogen electrode.

It was decided to make one determination with oxalate in the blood in the dialyser, and a second with the equivalent amount of calcium chloride added to it so as to produce coagulation. A blank experiment was first carried out to ascertain the effect of adding calcium chloride to the blood. Defibrinated goat's blood was taken and rotated in a tonometer containing alveolar air, at room temperature. After twenty minutes specimens were run into two dialysers, one of which contained 0·4 c.c. of 0·8 p.c. NaCl, and the other 0·4 c.c. of 4·8 p.c. $CaCl_2$. The p_H of each of these samples was found to be 7·33. Thus the addition of calcium chloride in this proportion does not appear to alter the reaction of the blood.

30 c.c. of goat's blood were drawn off into a vessel containing 0·7 c.c. of 10 p.c. potassium oxalate solution. 10 c.c. were rotated in alveolar air at room temperature and then three samples were drawn off into three dialysers: (1) containing 0·1 c.c. of 0·8 p.c. NaCl; (2) containing 0·05 c.c. of 4·8 p.c. $CaCl_2$; and (3) containing 0·105 c.c. of 4·8 p.c. $CaCl_2$. After dialysis it was found that the p_H of (1) and (2) was 7·35 and of (3) 7·36. The blood in the second dialyser was incompletely clotted, the blood in the third completely clotted.

The method was then varied and another determination made as follows. Specimens of cat's blood were drawn from the carotid artery direct into three dialysers, (1) containing a pinch of potassium oxalate, and (2) and (3) containing no oxalate. The p_H's were (1) 7·6; (2) 7·58; (3) 7·6—the variation in (2) being within the limits of experimental error. The blood in the first dialyser was not clotted, whilst that in (2) and (3) was firmly clotted.

From these observations it appears that the reaction of the blood is not affected by coagulation.

NOTE: The specimens of blood required for these experiments were obtained from the animals by Dr C. Lovatt Evans.

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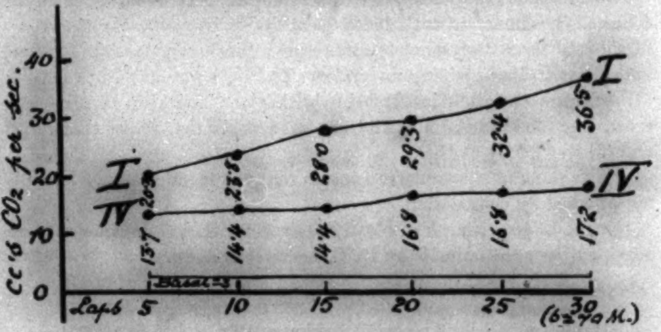
Physiological cost of walking—in and out of training. By A. D. WALLER and G. DE DECKER.

The object of the following observations was to measure the cost of a given amount of work done by a healthy adult, (*a*) out of training, (*b*) in training. We have been so fortunate as to enlist for this purpose the kind coöperation of Dr E. Lüscher (of Berne) who is an almost exclusiyely sedentary worker during the academic session, and a notably strenuous walker and climber during the vacation. The test-work carried out by him for our purpose in July when he weighed 62 kilos and was clearly out of condition, and in October after two months of rock climbing, when he weighed 66 kilos (clothed in both cases), consisted in a horizontal walk thirty times round a garden (209 × 30 = 6270 metres), *i.e.* of approximately four miles in one hour, at "go as you please" speeds in Observations I and II, at a restrained speed in Observations III and IV. The speed of walking was recorded on a revolving cylinder by a Marey tambour connected with a closed rubber tube upon which the subject stepped at each lap; a chronograph marked minutes. Samples of the expired air were collected during a space of 50 metres for a time of 25 to 28 seconds at every fifth lap, measured by a Boulitte spirometer, and titrated for CO_2. The readings were converted at once into c.c.'s CO_2 per second, and plotted as ordinates upon squared paper as shown in this graph of Observations I and IV.



As may be seen in the following summary of results, Dr L.'s "go as you please" speed is above the usual average 3½ to 3¾ m.p.h., *i.e.* 4¼ m.p.h. in his untrained state on July 1, and slightly over 4½ in his trained state on Oct. 22. And he was 4 kilos heavier on this second date. Yet the cost of work, or the CO_2/KgM ratio, was distinctly lower in the second case. At the approximately equal speeds 4·25 and 4·30 m.p.h. in Observations I and IV, the difference is surprisingly great, 0·215 c.c. per horizontal KgM in the untrained state as compared with 0·098 in the trained state.

Summary of Results.

Dr E. L. Cost of walking—in and out of training.

Observation	Speed proposed	Time of 30 laps (6270 m.)	Speed		Work KgM (hor.) per sec.	Cost of work c.c.'s CO_2 per sec. gross − basal = net	CO_2 c.c.'s/per KgM (hor.)	Calories per KgM (hor.) approximately at 5 cals. per 1 c.c. CO_2
			metres per sec.	(miles per hr.)				
I. July 1	"as you please"	55′ 0″	1·90	(4·25)	117·9	28·3 − 3·0 = 25·3	0·215	1·0
II. Oct. 22	do.	51′ 12″	2·04	(4·56)	134·7	28·0 − 3·0 = 25·0	0·186	0·9
III. Do.	restrained	59′ 52″	1·75	(3·91)	115·2	15·3 − 3·0 = 12·3	0·107	0·5
IV. Oct. 23	do.	54′ 20″	1·92	(4·30)	127·0	15·5 − 3·0 = 12·5	0·098	0·5

Further observations on the same lines are evidently required, and we should be very glad to obtain an opportunity of repeating the observations upon suitable volunteers, *i.e.* in and out of training, before formulating a conclusion concerning the efficiency of the human machine in and out of training.

Note.—We took a measurement of Dr L.'s efficiency by the staircase test on Oct. 24th with the following result:

$$CO_2 = 1\cdot25 \text{ c.c. per KgM}; \ \therefore \ \frac{KgM}{CO_2} = 0\cdot8 \text{ and Efficiency} = 32\ \%.$$

Radioactivity and smooth muscle. By E. Soref.

Spaeth (1) has shown the stimulating effect of KCl on the melanophores Fundulus Heteroclitus (that are functionally modified smooth muscle) and on the isolated uterus of the guinea-pig.

Potassium emits β rays (Rutherford). Therefore I investigated the action of other radio-active bodies. They all proved to be efficient. Uranium, thorium, and rubidium salts produced a submaximal contraction of the guinea-pig's isolated uterus immersed in Locke-Ringer, in smaller doses than that of potassium chloride. For cæsium a larger dose than that of KCl was necessary.

Also free radiations were effective. The proximity of a capsule of mesothorium produced an increased tonus of the uterus.

Zwaardemaker (2) in his attempt to substitute radio-active salts for KCl in frog's heart Ringer says he succeeded when using æquiradioactive doses.

In my experiments with the guinea-pig's uterus rubidium chloride acted as a perfect substitute; uranium and thorium were only partially successful.

Other smooth muscles were investigated. The intravenous injections into a pithed cat of rubidium and uranium salts and also of a solution of emanation produced a rise in the blood-pressure. The uterus and the intestine responded in the same way as they do to adrenalin. That is to say they have a sympathomimetic action—thorium and potassium proved to be fatal.

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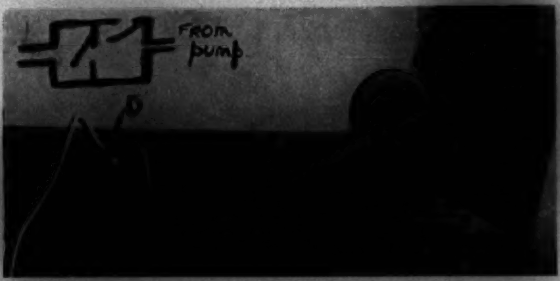
A simple artificial respiration apparatus. By J. W. C. GUNN.

Lack of a suitable apparatus for administering artificial respiration and the length of time that would elapse before one could be obtained from an instrument maker necessitated the improvising of some apparatus that would serve the purpose. That shown in the illustration was made out of oddments, and has proved very efficient.



The pump (*A*) was a pneumatic door-check. It is fixed to the base board by the hinged attachment at *B* (part of the pump as bought). The driving wheel, driven from rotating shafting by a continuous cord in the usual way, rotates the crank (*C*), which has three holes pierced in it. The piston rod of the pump is fixed to the crank by a screw which passes through a hole in the end of the rod and through one of the holes in the crank. This screw is rigidly fixed to the crank by a nut at each side, but fits loosely in the piston rod so as to allow for rotation. The stroke of the piston, and consequently the amount of air delivered, can be regulated by passing the screw through the different holes in the crank. The longest stroke is suitable for a dog, while the shortest is sufficient for a rabbit. A tube from the pump passes to a small box (*D*) containing two simple valves as shown in inset, arranged so that the air expelled from the pump is driven towards the animal but that no suction is made on the lungs when the pump is filling.

This pump was assembled for me by Mr R. McManus, mechanical assistant in the Physiology Department, University of Cape Town, at a cost of under £1. If fitted with a small motor driven from a wall-plug it would be found very useful for occasional theatre and other demonstrations where no other means of giving artificial respiration is installed.

The alleged hypersusceptibility of the rat to squill. By J. A. Gunn and R. St A. Heathcote.

That the rat is much more sensitive to the toxic action of squill than other laboratory animals is a statement which has long been accepted (1), and which is still current. We have not been able to find the original experiments upon which this statement is based. It is claimed that the use of squill as a rat poison depends upon it, because the rat succumbs to amounts of squill that are innocuous to rabbits, cats, dogs, pigs, fowls, etc.

It is well known, on the other hand, that the rat shows a high degree of tolerance to other glucosides of the digitalis group of which squill is a member. Thus one of us has shown (2) that the minimum lethal dose by subcutaneous injection of an extract of strophanthus was thirty times greater for the rat than for the rabbit. When the isolated hearts of these animals were perfused, it required about thirty times as strong a solution to arrest the rat's heart as sufficed to arrest the rabbit's heart in the same time. The congenital tolerance of the rat to strophanthus, is therefore, in part at least, due to a specific insusceptibility of the heart of this animal to the action of strophanthus.

It seemed remarkable, therefore, that the converse should be true of squill; and it seemed probable that, if the rat showed a congenital intolerance to squill but a congenital tolerance to the other members of the digitalis group, and if this were accompanied by a corresponding difference in toxicity to the heart, there must be some intrinsic difference between squill and these other glucosides in their action on the heart, and that this difference might be of importance in other connections.

Kopaczewski (3) stated that the rabbit, rat, etc., are about equally susceptible to squill administered subcutaneously, but when his data are reduced to dosage per kilogramme they show that the M.L.D. is slightly higher for the rat. He found that squill when ingested is more toxic to the rat than to other laboratory animals, but his experiments are not sufficiently complete to make this certain.

We have prepared a glucoside from squill by a modification of Ewins' process and have found that the minimum lethal dose per kilo by subcutaneous injection was about 5 mg. for the rabbit and 150 mg. for the rat. The rat, therefore, survives a dose thirty times greater than the rabbit. Also it requires much higher concentrations of the glucoside to arrest the rat's heart than suffices to arrest the rabbit's heart in the same time. In so far as this glucoside is concerned, therefore, squill resembles strophanthus; the rat's heart is relatively insensitive to both.

It is of course still possible that the rat may be more susceptible than other domestic animals to squill taken by the mouth; but this, if true, must be due either to some constituent of squill other than the glucoside or to some difference in action other than the action on the heart. The possibility that this alleged hypersusceptibility of the rat may be due merely to the fact that the rat is the only domestic animal that will eat squill has not been sufficiently excluded.

The object of this note is to point out that, so far as concerns the lethality by subcutaneous injection and the toxic action on the heart by perfusion, the glucoside of squill that we have used resembles the other glucosides of the digitalis group. The rat is markedly *less* susceptible than the rabbit.

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The sympathetic innervation of the vagina. By J. A. Gunn and D. S. Davies.

Three types of uterine innervation are known: (*a*) as in the rabbit, where the sympathetic nerve is motor to the uterus whether pregnant or non-pregnant (1); (*b*) as in the cat, where the sympathetic nerve is motor to the pregnant but inhibitor to the non-pregnant uterus (2); and (*c*) as in the guinea-pig, where the sympathetic nerve is inhibitor to both pregnant and non-pregnant uterus (3). The reaction in these cases to adrenaline is qualitatively identical with the effect of sympathetic stimulation. The effect of sympathetic stimulation of the vagina has not been revised with a view to determining whether it follows or differs from that of the uterus in the case of the three types above mentioned.

Where the effect of sympathetic stimulation of the uterus is motor the coexistence of inhibitor fibres can be shown by special methods. The influence of the latter is usually masked by the more powerful effect of the motor fibres when the mixed sympathetic nerve is stimulated. The existence of a parasympathetic nerve supply to the uterus is denied.

We have so far investigated chiefly the reaction of vaginal muscle to adrenaline, using this as an indication of the effect of sympathetic stimulation, and have found that it is motor in every case, regardless of what may be the reaction of the uterus. Thus the vagina of the non-pregnant cat, of the guinea-pig and of the rat contract under the action of adrenaline just as does the vagina of the rabbit. A motor effect is obtained with adrenaline whether one records the movements of the longitudinal or circular muscles of the vagina. If a record be taken of a series of rings of the vagina, the motor effect produced by adrenaline is greater on those rings nearer the vulvar orifice, but the effect does not seem to be restricted to a sphincter vaginæ. The motor effect on the vagina is quantitatively much less than the inhibitor effect on the uterus, so that when a record is taken of the combined movements the former factor is easily missed.

There is evidence that the vagina contracts during coitus. This contraction may therefore occur either through the parasympathetic (sacral) nerves(4) or through the sympathetic. In animals like the cat, guinea-pig, or rat, a purely sympathetic stimulation, giving rise to a contraction of the vagina with relaxation of the uterus, may conceivably aid in the injection of spermatozoa into the cavity of the uterus.

It is possible that the motor effect of sympathetic stimulation on the vagina may be found to be more pronounced during the œstrous period.

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PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY,

December 18, 1920.

Agglutination by Ricin, etc. By J. A. GUNN.

In the course of experiments instituted with a view to seeing if it might be possible to discover whether any particular constituent of the red blood corpuscles was specially concerned in agglutination by ricin, I found that ricin "agglutinated" a suspension of cholesterin. The cholesterin suspension was made by dissolving cholesterin in ether and triturating the solution while the ether evaporated; the resulting cholesterin powder was shaken with water, allowed to stand for a few days till the larger particles deposited, and the supernatant suspension was then very permanent. One such suspension has not completely deposited in two years.

In order to obtain a suspension more nearly resembling blood in size and weight of particles, I poured a solution of cholesterin dissolved in ether over finely powdered carmine and stirred while the ether evaporated, with the intention of coating the carmine particles (which are insoluble in ether) with a film of cholesterin. A carmine-cholesterin suspension of this kind in water was also agglutinated by ricin. I then found, however, that a carmine suspension without cholesterin was also agglutinated by ricin, as was a suspension of resin. It was obvious, therefore, that this agglutination was not chemical but physical, seeing that the suspensions could hardly have anything in common beyond the possession of a negative charge on the suspended particles. As the red blood corpuscles were also known to have a negative charge, the question arose whether the agglutination of red cells by ricin was not similar in type to the agglutination of other suspended particles which possess a negative charge. Of a large number of experiments dealing with these questions I wish to mention some of the results.

The accompanying photograph shows the result of passing a current simultaneously through two Hardy's tubes in series, the one containing a cholesterin suspension in distilled water, the other (on the right facing) a cholesterin suspension agglutinated by ricin also in distilled water, both suspensions being layered under distilled water. When the current passes the non-agglutinated particles migrate to the positive pole, but the agglutinated particles remain stationary. Their agglutination was, therefore, accompanied by an abolition of the negative electric charge of the particles and the presumption was that ricin acted as a positively charged colloid, and that the agglutination was an example of Hardy's rule.

Fig. 1.

The cholesterin suspension showed towards electrolytes the usual behaviour of a suspension of negatively charged particles, being precipitated in 24 hours by molar solutions in concentrations of 0·35 NaCl, 0·53 KCl, 0·009 $CaCl_2$, 0·009 $BaCl_2$. The precipitating power of ricin towards such suspensions was enormously influenced by the presence of electrolytes, *e.g.* a carmine-cholesterin suspension was precipitated in 24 hours by ricin 1 in 2000 in distilled water, and by 1 in 25,000 when sodium chloride was present in 0·25 per cent. concentration.

Experiments similar to that in Fig. 1 were performed with rabbit's corpuscles. I found that when a current is passed through two Hardy tubes in series, the one containing a suspension of red blood corpuscles in isotonic sugar solution, the other red blood corpuscles agglutinated by ricin also in isotonic sugar solution, both layered under isotonic sugar solution, the non-agglutinated corpuscles migrate away from the negative pole but the agglutinated do not. In the process, therefore, of agglutination by ricin, the red cells lose their negative charge. The conclusion drawn from these experiments is that agglutination by ricin is partly at least non-specific and is of the nature of the precipitation of one colloid by a colloid of opposite sign. It does not follow that this is the sole factor concerned in ricin-agglutination of red blood cells.

In further experiments I found that cobra venom and Daboia venom agglutinated a cholesterin suspension in concentrations of 1 in 100,000, Echis venom in 1 in 5000, but dried egg albumen did not agglutinate in 2000. Cobra venom hæmolysed red cells in concentration of 1 in 30,000, echis venom did not hæmolyse in 1 in 2000. The samples of venom were old, but the cobra and daboia venoms were still toxic as tested on rabbits, whereas the echis venom had nearly completely lost its activity. So far as these experiments go—and in the meantime they are merely provocative—they suggest a relation between the physiological activity of toxins and their capacity to act as positively charged colloids.

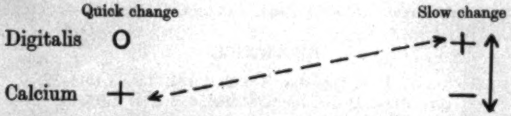
Note on antagonisms and reinforcements. By W. Burridge.

The author has shown that cardiac excitability can be reversibly influenced in two ways, (1) quick changes coming and going concomitantly with the substance producing them, (2) changes slower developing and especially subsiding slowly. On the basis of such changes a table was constructed some time ago taking calcium as the standard. A negative sign indicates that the magnitude of the cardiac response to calcium is diminished or, expressed differently, a greater amount of calcium is now necessary to evoke contractile activity from a given proportion of the whole contractile material and vice versa for the positive sign. The sign for the quick change produced by barium has been placed in brackets because regarded in terms of excitability alone its quick change is positive since it then acts as a substitute for calcium, but inasmuch as in doing so it displaces and antagonises calcium this action from the calcium standard is negative.

Substance	Quick change	Slow change
Adrenin	–	+
Alcohol	–	+
Barium	(+)	–
Caustic soda or potash	–	+
Chloral hydrate ...	–	+
Chloroform	–	+
Digitalis	0	+
Ether	–	+
Magnesium	–	+
Potassium	–	+
Sodium	–	+
Calcium	+	–

This table has been used for predicting the existence of relations between its elements. For example, make the short abstract of digitalis and calcium.

We have

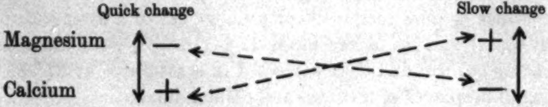


We have (1) a direct relation of antagonism between their slow changes indicated by the double arrow, (2) an indirect relation of reinforcement indicated by the double broken arrow.

The indirect relation of reinforcement was discovered by Clark (1) who found that the assumption of the systolic state by a heart under the influence of digitalis depended on calcium. His experiments did not lead him to look for an antagonism. This table shows there is an antagonism of which the experimental verification is given elsewhere (2).

The relations between magnesium and calcium are more complicated.

We have



There are two groups of relations:

1. Their direct relations—indicated by the double upright arrows whereby they produce excitability changes of similar type but to opposite ends.

2. Their indirect relations—indicated by the double broken arrows whereby they produce excitability changes of different type to similar ends.

The relations between magnesium and calcium are thus fourfold, viz. two direct antagonism between excitability changes of similar type and two indirect reinforcements between excitability changes of different type. Many observers have described an antagonism between magnesium and calcium but not the existence of two antagonisms or the crossed reinforcements. The predictions made from this table have been verified.

The finding of the two modes of excitability changes with the corresponding direct and crossed reinforcements and antagonisms renders the subject more complex at first but eventually leads to simplification. Thus, the controversy as to whether sodium or calcium initiates the heart beat has a solution from the table in a crossed reinforcement whereby sodium winds up the excitation spring until it is released by the calcium catch.

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The absorption curve of hæmoglobin and carbon dioxide. By GEORGE A. BUCKMASTER.

The curves given by Bohr in 1886 were for solutions of hæmoglobin of a strength of 1·76 p.c. and 3·8 p.c. These were not reproduced in his account of the blood gases in 1905 in Nagel's *Handbuch der Physiologie*.

I have determined about 25 points for the absorption of carbon dioxide by hæmoglobin (horse) between pressures from 0 to 98 mm. of CO_2 and find that solutions of hæmoglobin behave towards this gas according to the Dalton-Henry law.

Solutions of pure recrystallised hæmoglobin of a strength approximately equal to that in the blood 12·8–11·9 p.c. were first rendered completely gas-free and then subjected in a saturator at 37°–38° C. to varying atmospheres of nitrogen and carbon dioxide.

Freshly made solutions were used for each experiment, and, except that the 25 c.c. of solution used was evacuated in the pump, the whole procedure followed that employed by Christiansen, Douglas and Haldane[1]. The volume of the saturator was 2500 c.c. so that the relative volumes of solution and gas-space were nearly the same as in their experiments. Where the object was to ascertain that a given solution absorbing a definite volume of carbon dioxide at a known pressure, dissociated again at a lower pressure twice the volume of hæmoglobin was used.

Solutions of hæmoglobin do not absorb quite so much carbon dioxide as a deposit of red corpuscles of the same strength in hæmoglobin, but the difference is small. The curve is lowered should any of the hæmoglobin become oxy-hæmoglobin. Between 40 and 60 mm. pressure of CO_2 considerable quantities of carbon dioxide are absorbed by hæmoglobin, and at any given pressure of CO_2, the amount of gas taken up is lost rapidly on exposure to a lower pressure.

Absorption of carbon dioxide by hæmoglobin solutions of 12·8 p.c.

Time in saturator in minutes	Pressure of CO_2 in saturator in mm. Hg.	Vols. of CO_2 per 100 c.c. Hb solution
65	7·08	5·84
60	13·8	7·65
64	17·1	10·1
60	27·1	14·52
62	38·1	16·1
64	47·1	19·9
63	54·4	24·46
62	58·0	26·2
64	76·2	28·9

Capillary pressure (II). By Leonard Hill.

In the *Proc. Physiol. Soc.* July 10, 1920, I pointed out that, using the method of Roy and Graham-Brown, the pressure which stops the flow does not indicate the capillary pressure, but that in the arteries which supply the area compressed, and that the capillary pressure may be taken to be that pressure which just begins to lessen the velocity of flow in those capillaries wherein the flow is slowest; these naturally first undergo compression, the blood taking the pathway of less resistance through other capillaries. If the compression be gradually increased the flow is slowed, then stopped in more and more of the capillaries until it continues only through the most direct pathways connecting the arterioles with the venules, wherein the velocity was greatest to start with. With further increase of compression the flow of blood becomes pulsatile not only in the arterioles but in these capillaries, and in the venules, the compression being sufficient to stop the flow in diastole, but not in systole. Finally the blood is expressed out of the capillaries and veins, and the pulse is seen swinging the corpuscles to and fro in the arterioles, but not translating them onwards; the pressure required to just produce this effect is the systolic pressure in the arteries supplying the compressed area.

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[1] *Journ. of Physiol.* 48. p. 241. 1914.

It was pointed out that all those phenomena which take place on the application of pressure to the arm by means of the cuff of the sphygmo-manometer can thus be observed. The diastolic pressure can be measured in the frog's web. It is that pressure which just arrests onward flow of the corpuscles in diastole, while the systolic is that pressure which just prevents onward flow in systole. For example in a frog's web these pressures were 23 and 36 cm. of water.

To determine the pressures correctly, the transparent membrane covering the compression chamber must be tied on loosely, as Roy and Graham-Brown direct, so that when blown up it forms a dome over which the web is spread, the threads attached to the toes being carried to pins, and the leg arranged so that the flow of blood to the web is free and unobstructed. The glass against which the web is pressed is lowered until it touches the web and toes on either side, the top of the dome being thereby slightly flattened. The web is compressed between the flattened part of the membrane and the glass. Great care must be taken to secure that the toes are not pressed against the top of the pressure chamber, and that pressure is not spent in merely expanding the membrane.

If compression be momentarily applied to webs so arranged, it will be found that a pressure of 2–5 cm. of water momentarily checks the flow of corpuscles in the arterioles, while it takes some 20–35 cm. of water to stop the flow altogether, that is in the case of frogs with the brain destroyed, or under urethane. The lower the arterial systolic pressure the less is the pressure required momentarily to check the flow. In the chief capillary branches, where velocity of flow is rapid, the same pressure momentarily checks the flow, and at the same time as in the arteriole, but in the case of a fast flowing venule the flow may be accelerated during and retarded momentarily after a momentary application of compression. The acceleration is due to the expression of blood from the capillaries, the retardation to the capillaries filling again. To slow flowing venules a pressure of 0·5 to 1 cm. of water, momentarily applied, may suffice to check the flow. The pressure which momentarily checks the flow in the arteriole is the true pressure and closely approximates to that pressure which, when continuously applied, retards the flow in those capillaries with the slowest rate of flow, *e.g.* those seen at the very edge of the web. The check is momentary, because if the compression is maintained the pressure banks up behind it.

As the water manometer, owing to its inertia, does not fully record the value of a momentary compression, I have used an air manometer with a xylol index, graduating this against the water manometer.

The check-flow index is quite sharp, for example a pressure of 3 cm. H_2O momentarily applied may not check the flow momentarily in the arterioles, while a pressure of 4 cm. H_2O may do so. The conclusion then is reached that the pressure in the arterioles with unobstructed rapid flow, is not higher than about 2–5 cm. of water, and that the fall of pressure from arteriole to venule is a very slight one.

Observations have been made on the mouse's ear and mesentery, and the cat's mesentery, which confirm the conclusion that the pressure in the capillaries, arterioles and venules is no higher than in the case of the frog's web. The assumption which has been made that the capillary pressure is some 30 mm. Hg and the hypotheses as to the transudation of lymph, urine, etc., based on that assumption appear then to be untenable. Be it noted that an area 2 mm. in diameter in the frog's web includes capillaries, arterioles and venules, and that the measurement of the pressure required to pale an area of skin in man is a measurement not of capillary pressure, but of the pressure in the small arteries supplying that area. The readings are also too high owing to the resistance of the horny layer of the epidermis.

Note on a retinal reflex in the frog following benzene administration. By A. T. Cameron and F. A. Sedziak.

We can find no reference in the literature to the reflex described below and as through pressure of other work we are unable to investigate it further, we have decided to publish this note, since the observation appears to present several points of interest meriting further investigation. It was noted incidentally in rehearsing a class experiment on the relative pharmacological effects of different hydrocarbons.

Injection of 0·5 c.c. of pure benzene into the anterior lymph sac of a frog weighing about 50 grams produces distinct effects within a few minutes. The eyes close, movements become sluggish, when turned over the animal recovers its normal position with difficulty, and in most cases can no longer do so after ten minutes. The body often becomes characteristically arched. The usual reflexes gradually disappear. Breathing ceases. With the gradual disappearance of the conjunctival reflex the eye opens, the nictitating membrane is usually drawn down, and the "retinal" reflex can be observed. Initially it would appear to be evoked by any change in intensity of illumination. When the hand is passed suddenly between the frog's eye and the source of light within one or two seconds the head is moved, and usually the hind limbs also. When a bright light (electric light at a distance of one foot) is suddenly switched on, the response follows. On switching the light off the response is more marked. Direction of light to and from the pupil only, the rest of the head being screened from light changes, evokes the response. Gradually the response to sudden increase of illumination ceases. The response to sudden decrease of illumination persists much longer. Cessation of stimulus appears therefore to be more powerful than application of stimulus. Ether anaesthesia abolishes the response to increase of illumination first.

The maximum effect is reached at from twenty to twenty-five minutes after injection, and the reflex disappears in another twenty to sixty minutes or, occasionally, after even a longer interval. Quality of light does not affect the result; both red and blue light evoke the reflex. Intensity of light appears to be the governing factor. A retina fatigued by the shining of a strong light upon it for some minutes does not call forth such an active response on switching off the light. There may be no movement, but after a minute or two the response can be again elicited by further light stimulation. The degree of response varies in different animals from a slight head movement to such vigorous movements of the hind limbs as would be excited by a strong electric current applied externally. After the reflex has disappeared electrical stimulation still produces active muscular responses. Examination of the animal at this stage shows a very dilated heart, auricle still beating, ventricle stopped, lungs collapsed. There is always marked red pigmentation of the abdomen and hind limbs, which develops a few minutes after injection of the drug.

The optimum dose of benzene appears to be 1·2 per cent. of the body weight. Two per cent. induces convulsions and death rapidly, and the retinal reflex cannot be observed, while 0·7 per cent. may not produce any definite effect. In one experiment, in which no marked effect on the frog had been observed during one hour after injection of this dose, subsequent injection of a 0·4 per cent. dose evoked an active response in twenty minutes.

Similar effects have been observed with toluene and to a less extent with phenol. Chlorbenzene and brombenzene give the reflex when injected in somewhat larger doses. The reflex could not be detected after injection of similar doses of xylene, aniline, nitrobenzene, benzyl alcohol, and pyridine.

These experiments have been carried out on *R. pipiens*.

Influence of the vagus upon the refractory period of the dog's auricle[1]. By A. N. Drury, T. Lewis and H. A. Bulger. (*Preliminary notice.*)

Two secondary coils are joined in series with each other and with a pair of stimulating electrodes. The latter are attached permanently to a chosen point of the muscle of the right auricle. From one secondary coil rhythmic break shocks are sent into the muscle at a rate of approximately 200 per minute; these shocks maintain the rate of the auricular beats at a constant level throughout the experiment. From the other secondary coil occasional shocks are sent in and these shocks test the length of the refractory period of the muscle which is responding to rhythmic stimulation. It is a matter of importance that these test shocks should lie well above threshold value; it is also important in estimating the refractory period of the muscle that the testing shocks should enter the muscle at the point at which its rhythm is originating. It is for this reason that the two series of shocks are sent through the same electrodes. The responses of the heart are recorded by means of the string galvanometer connected to a pair of non-polarisable contacts placed on the muscle at a little distance from the stimulating electrode. The shocks are recorded by the second string of the instrument. Very accurate readings are obtained by measurement of the plates obtained. Observations have been made upon eight dogs, fully anæsthetised with morphia, paraldehyde and ether.

When the auricle is beating at rates in the neighbourhood of 200 per minute, the refractory period measures in the average about 0·06 of a second; under vagal stimulation, sufficiently powerful to bring the ventricle to a standstill, it measures in the average about 0·02 of a second; in the fully atropinized animal it measures in the average about 0·12 of a second. The relatively low readings (0·06) in the unatropinized animal is due to a local stimulation of the vagus fibres in the heart by the rhythmic shocks; the full reduction of the refractory period of which the vagus seems capable is represented by the difference in the values 0·12 and 0·02 of a second. The reduction is to about one fifth or one sixth of the full value. In the atropinized auricle beating at 200 per minute (cycle of 0·3 of a second) the period of responsiveness measures about 0·18 of a second (60 p.c. of the cycle); under full vagus influence it measures about 0·28 of a second (93 p.c. of the cycle). Thus under vagal stimulation the auricle becomes responsive to electrical stimulation over a much larger proportion of its cycle; this observation is scarcely paradoxical, since the length of the refractory period varies in the same direction as does the length of the contraction process, and vagal stimulation has a profoundly weakening and shortening influence upon the contractions of the auricle.

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[1] Work undertaken on behalf of the Medical Research Council.

The action of carbon dioxide on the frog's heart. By W. BURRIDGE. (*Preliminary communication.*)

The present author has already shown that the fresh heart of the frog perfused with the usual Ringer's solution is resistant to change and ill-adapted to show delicate augmentation. Successful endeavours have now been made to render the perfused heart more responsive to change and a preparation obtained which is not less than 500 times more responsive to the augmentive change of carbon dioxide than the one customarily employed by physiologists. This preparation shows:

1. That carbon dioxide in small doses exerts a marked augmenting action on the activity of the heart of the frog.
2. That the augmenting action has many resemblances to the action of adrenin.
3. That oxygen is necessary to this augmenting action.
4. In absence of oxygen an amount of carbon dioxide that would otherwise excite can depress.
5. That carbon dioxide does not act in virtue of its concentration in hydrogen ions.
6. That the endocrine glands can determine the degree and nature of the response of an organ to carbon dioxide.

Effect of vagus upon the rate of transmission of the excitation wave in the dog's auricle[1]. By T. Lewis, A. N. Drury and H. A. Bulger. (*Preliminary notice.*)

Some years ago one of us, working in conjunction with Meakins and White (*Phil. Trans.* 1914, ccv. 375) obtained evidence that vagal stimulation (right and left) has no influence upon the rate at which the natural excitation waves are propagated through the dog's auricle. These results were complicated in certain degree by a lesser or greater reduction of the rate of the auricular contractions concurrent with the rise of vagal tone, though it seemed clear that this reduction in rate did not invalidate the conclusion. It has seemed desirable nevertheless to test the question again, maintaining the auricular rate at a constant point in and out of vagal stimulation by rhythmic stimulation.

The auricle is stimulated by means of rhythmic break shocks in line with two pairs of non-polarisable contacts laid on the auricle, each pair of the latter being connected to one string of the recording galvanometer. The rate at which the excitation waves are propagated is indicated by close measurement of the interval separating corresponding deflections of the two strings. The effect of stimulating the right (or left) vagus[2] upon the right auricle has been examined in observations upon twelve dogs, fully anæsthetised with morphia, paraldehyde and ether. The rate of transmission has been tested over the body of the right auricle, and over the tænia in the region of superior and inferior vena cava. Using rates of rhythmic stimulation lying between 180 and 300 per minute we find that vagal stimulation, sufficiently powerful to bring the ventricle to a standstill, is without effect upon the rate of transmission.

Thus, we have been unable to find in the dog's auricle any effect comparable to that which seems evident from certain of Gaskell's well-known observations upon the tortoise auricle, namely, evidence of depressed conductivity under vagal stimulation. The contrast between the two series of observations is sufficient to emphasize the need of caution in arguing from the cold-blooded to the warm-blooded auricle; our observations are also of interest in showing that, while vagal stimulation profoundly affects conduction at the *A-V* ring of the dog, a similar effect on the auricular muscle is lacking.

If the dog's auricle is stimulated at rates *surpassing* 300 per minute, the transmission intervals in the auricle become irregular and are in the average a good deal prolonged above those obtaining at lower rates. In such circumstances vagal stimulation *reduces* the transmission intervals approximately to the values prevailing at lower rates of stimulation, and abolishes the irregularity. We ascribe these two effects of vagal stimulation to its power to reduce the refractory period of the auricular muscle, and conclude that lengthened and irregular transmission intervals in the dog's auricle result when the oncoming excitation waves flow through muscle in which the refractory phase has not completely subsided, a condition brought about when the rate of stimulation is sufficiently advanced.

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[1] Work undertaken on behalf of the Medical Research Council.

[2] The right nerve has received chief attention.

There seems to us to be sufficient evidence that the lengthened and irregular transmission interval in auricles responding to high rates of stimulation, described by Lewis, Feil and Stroud (*Heart*, 1920, VII. 247), and those observed in instances of auricular flutter, whether the latter be pure (*ibid.* p. 191) or impure (*ibid.* p. 293), are of this nature.

Self-adjustment of blood-pressure. By W. A. Osborne.

In 1916[1] I pointed out that as all self-adjusting mechanisms must produce an oscillatory equilibrium, we ought to expect such in a number of standards maintained in the body. The generalisation might perhaps be made that the amplitude of the oscillation should, in health, be too small to be easily detectable and should become prominent only in pathological states. A striking instance has been furnished by Haldane and his school in elucidating the nature of Cheyne-Stokes respiration. We find oscillatory adjustment in temperature regulation and also, I contend, when a limb (including the eye) assumes through voluntary muscular action a special directive position not an extreme of joint movement.

It is well known that a rise of blood pressure by stretching the aorta stimulates the depressor vagus fibres ending in it, and causes reflexly a slowing of the heart beat. It is known also that a fall of blood pressure will quicken the heart beat. It seemed possible that the lessening of aortic tension might stimulate vagus fibres different from those stimulated by stretching, and cause reflexly a quickening of the beat. The two kinds of nerve fibres would then be part of a self-adjusting mechanism. In order to test this, the right vagus of a dog under morphia was cut; the longitudinal surface and the cut end of the distal part were connected with a string galvanometer, and the excursions of the string observed by the shadow cast on a screen. There was a tremor of the string, and as has been described by others, there was an excursion at the time of greatest lung inflation, and none at the end of expiration. Raising the blood pressure by clamping the abdominal aorta caused a movement of the string, and on unclamping the aorta, the string returned to its previous position.

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[1] Oscillatory Adjustments in the Animal Body. *Proc. Roy. Soc. Victoria*, **29**. New Series. Pt. 2, p. 115. 1916.

This movement was still better displayed on injecting intravenously 5 c.c. of 1 in 50,000 adrenaline solution. The return of the string to its normal position took some minutes and so far as the eye could judge altered parallel with the carotid blood pressure. On opening the cannula in the carotid artery and letting profuse hæmorrhage take place, the string moved more quickly back from the position caused by high pressure.

In a second animal the abdominal aorta was first clamped and then cut; the results were similar to those in the first experiment and in addition the sudden lowering of blood pressure caused an immediate reduction of the tremor of the string. From these results it appears that whilst stretching the aorta sets up impulses in the depressor nerve fibres, the lessening of the stretching simply lessens the stimulation, and does not give rise to additional nerve impulses.

It is known that section of the depressor in the rabbit does not abolish vagus tone. Few observations appear to have been made on the immediate effect of section of the depressor. Bayliss(1) found no effect on blood pressure. He quotes Ludwig and Cyon as also having found none, and Sewall and Steiner as having found a rise of 1–3 mm. Hg. I have made a number of experiments on this point and obtained a quickening of the heart beat and some rise of blood pressure, so that I think the self-adjusting mechanism of the depressor is in constant action.

REFERENCE.

(1) Journ. of Physiol. 14. p. 314. 1893.

PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY,

January 22, 1921.

Anoxæmia and the administration of oxygen. By D. K. Adams and N. Morris. (*Preliminary communication.*)

I. *The head of oxygen in arterial blood in anoxæmia.*

In order to come to definite conclusions as to the value of the administration of oxygen in cases of anoxæmia, further information on several points is required.

In the first place, it is essential to know how far the oxygen saturation of the arterial blood may fall and still allow of an adequate supply of oxygen to the tissues as indicated by the de-saturation of the venous blood. The distribution of oxygen in the arterial and venous blood in pneumonia has been studied by Stadie(1) who performed puncture of the radial artery in a series of cases of that disease. He found that the oxygen contents of the arterial was always about 10 per cent. to 15 per cent. over that of the venous blood thus indicating that an adequate supply of oxygen to the tissues is maintained even in anoxæmia of a considerable degree.

We have reinvestigated the question by decreasing the oxygen supply to the lungs and blood in various ways: (1) by closing the trachea, (2) by causing collapse of one lung by the production of a pneumothorax, (3) by occluding one bronchus.

The oxygen content of the arterial and venous bloods was estimated in these various conditions.

Methods. (1) Closing the trachea. Cats under ether anæsthesia were used; a tracheal tube inserted and the carotid artery and the jugular vein exposed. The jugular vein was selected as coming from an area in which the alterations in peripheral circulation are less modified than in other parts of the body. The blood was drawn off into all-glass 5 c.c. syringes. A small quantity of liquid paraffin was employed to exclude air. To ensure synchronicity in obtaining arterial and venous blood it was subsequently found desirable to insert a small arterial cannula from which the blood was collected under paraffin by one operator, while the other simultaneously performed venous puncture. Asphyxia of various degrees was subsequently induced in successive experiments by clamping the tracheal tube.

(2) Collapse of one lung. The thoracic wall on the right side was opened and the collapse of the right lung demonstrated by digital examination.

(3) Occlusion of bronchus. Dogs were employed. A large plug of cotton-wool soaked in oil was inserted into the trachea and by means of a probe was pushed into one bronchus. It was found impossible to ascertain the exact position of the obstruction until the post-mortem examination. In every experiment the plug was found to be in the left bronchus.

The oxygen analysis of the blood was carried out by Barcroft's differential method.

Results. (1) *Closing the trachea.* As asphyxia advanced down to a certain point the amount of oxygen in the arterial blood remained greater than in the venous, but in varying proportions, *i.e.* a "head of oxygen" was maintained. After a certain critical point was passed the oxygen saturation in the arteries fell more rapidly than in the veins, and in the later stages of asphyxia the venous blood contained a higher percentage of oxygen than the arterial. The critical point has not been ascertained owing to the difficulty in estimating accurately the degree of asphyxia induced. It would appear to lie somewhere about an arterial de-saturation of 80 per cent.

Number of experiment	Degree of asphyxia induced in stage 2	Percentage arterial and venous unsaturations			
		Before induction of asphyxia		After induction of asphyxia	
		Arterial	Venous	Arterial	Venous
2.	+	18·2	31·9	29·5	52·2
5.	+ +	5·0	50·0	28·0	71·9
3.	+ +	4·7	45·0	53·0	81·8
1.	+ + +	5·0	38·0	82·4	77·1
4.	+ + +	4·7	46·0	81·0	68·7

(2) Collapse of one lung suddenly induced (4 Exps.) produces a marked fall in the oxygen content of the arterial and venous bloods, the fall being permanent.

(3) Occlusion of one bronchus (3 Exps.) produces a marked fall in the oxygen saturation of the arterial and venous bloods.

To determine how far the blood of the jugular vein is representative of the total venous blood returned to the heart, experiments were performed with the thorax opened and with artificial respiration, in which blood was drawn simultaneously from the jugular vein and right ventricle.

The results show that the de-saturation is always less in the vein than in the ventricle.

To determine the extent to which the condition of the peripheral circulation may modify the oxygen content of venous blood the effect of temperature of the part on the de-saturation of the venous blood was studied. One leg of a cat was kept in hot water, the other immersed in ice, and blood was drawn off simultaneously from the femoral veins.

The venous blood in the warm leg contained about 20 per cent. more oxygen than did the venous blood from the cold leg. In one experiment in which the bloods were drawn off while the animal was in a state of partial asphyxia the difference was still present but not so marked.

Conclusions.

1. There is a gradual fall in the arterial oxygen saturation as anoxæmia increases.

2. The fall in the venous oxygen is not so strictly proportional to the fall in the arterial, as indicated by Stadie.

3. The irregularity of the venous curve may depend upon a variety of causes of which one is the state of the circulation in the part from which the venous blood is taken.

4. At a certain point (about an arterial de-saturation of 80 per cent.) the oxygen content of the venous blood becomes greater than that in the arteries.

The expenses of these series of experiments were defrayed by a grant from the Medical Research Council at whose instance the work was undertaken.

REFERENCE.

(1) **Stadie.** Journal of Experimental Medicine, 30, No. 3

The influence of cod-liver oil and butter fat on the retention of calcium and phosphorus.

By S. V. TELFER. (*Preliminary communication.*)

Rothberg, Steinitz, Meyer, Freund, and Orgler record experiments tending to show that an increase of milk fat in the diet decreases the retention of calcium, either by decreasing its absorption or by increasing its elimination by the bowel.

Orgler quotes some results which appear to indicate that the addition of cod liver oil increases calcium retention, and Freund records an observation showing that sesame oil acts in the same way.

The surprising differences in the effects of these fats seemed to deserve further consideration, and a series of observations were made on the retention of calcium and phosphorus in a normal child, for the purpose of comparing the effects of cod liver oil and butter fat.

The subject, B. C. aged 8 months, was put on a metabolism bed, and the fæces and urine collected separately.

During the first period the diet consisted of 200 c.c. of ordinary cow's milk, given five times daily for five days (21·6 gms. fat daily). At the end of this period 6 gms. of fresh butter were added to each 200 c.c. milk (43·2 gms. fat daily). Finally, during the third period the diet was altered to skim milk, to each 200 c.c. of which 6 gms. of cod liver oil were added (7·2 gms. milk fat + 21·6 gms. cod liver oil daily).

A half drachm of cane sugar was given in all the feeds.

During the last five days of the three periods the urine and fæces were collected quantitatively daily. There was no loss of excreta and no vomiting occurred during the course of the observations. The intakes of calcium and phosphorus were the same throughout.

The initial weight of the child was 14 lb. 10 oz. At the close of the last period it was 15 lb. 1 oz.

Period I. Cow's milk (daily intake 21·6 *gms. fat).*

			Intake		Output	
Day	Urine c.c.	Fæces (dried) gms.	CaO gms.	P_2O_5 gms.	CaO gms.	P_2O_5 gms.
Sept. 7th	610	10·4	1·7	2·2	1·38	1·74
„ 8th	600	12·3	1·7	2·2	1·51	1·88
„ 9th	630	15·8	1·7	2·2	1·36	1·90
„ 10th	640	16·2	1·7	2·2	1·47	2·17
„ 11th	560	11·0	1·7	2·2	1·04	1·76
	Totals		8·5	11·0	6·76	9·45

Five days retention CaO 1·74 gms. P_2O_5 1·55 gms.
Daily average retention CaO 0·34 gm. P_2O_5 0·31 gm.

Period II. Cow's milk (daily intake 43·2 gms. fat).

			Intake		Output	
	Urine	Fæces	CaO	P_2O_5	CaO	P_2O_5
Day	c.c.	gms.	gms.	gms.	gms.	gms.
Sept. 20th	530	13·7	1·7	2·2	1·28	1·70
„ 21st	460	13·8	1·7	2·2	1·09	1·75
„ 22nd	390	15·6	1·7	2·2	1·31	2·3
„ 23rd	460	19·5	1·7	2·2	1·53	1·90
„ 24th	520	12·6	1·7	2·2	1·33	1·92
	Totals		8·5	11·0	6·54	9·57

Five days retention CaO 1·96 gms. P_2O_5 1·43 gms.
Daily average retention CaO 0·39 gm. P_2O_5 0·28 gm.

Period III. Skim milk (daily intake 7·2 *gms. milk fat* + 21·6 *gms. cod liver oil*).

			Intake		Output	
	Urine	Fæces	CaO	P_2O_5	CaO	P_2O_5
Day	c.c.	gms.	gms.	gms.	gms.	gms.
Sept. 28th	430	15·1	1·7	2·2	1·44	1·96
„ 29th	510	13·8	1·7	2·2	1·23	1·79
„ 30th	640	15·7	1·7	2·2	1·49	2·29
Oct. 1st	450	13·6	1·7	2·2	1·20	1·86
	Totals		6·8	8·8	5·36	7·90

Four days retention CaO 1·44 gm. P_2O_5 0·90 gm.
Average daily retention CaO 0·36 gm. P_2O_5 0·22 gm.

Orgler's claim that butter fat and cod liver oil in the diet specifically affect calcium retention receives no support from these results. Practically no differences in calcium retention were found to occur from the variations made in the nature of the fat.

Anoxæmia and the administration of oxygen. By D. K. Adams and N. Morris. (*Preliminary communication.*)

II. *Effect of administration of oxygen upon the anoxæmia produced by (a) pneumothorax, and (b) occlusion of one bronchus.*

Methods. As in last communication.

(*a*) Unilateral pneumothorax was induced and subsequently oxygen was administered for a period of 5 minutes by allowing it to pass down the tracheal tube under very low pressure.

The arterial and venous anoxæmia induced by collapse of one lung from unilateral pneumothorax is removed by the administration of a small quantity of oxygen. The return of the arterial oxygen saturation to normal is rapid and practically complete under these circumstances, although only one lung is functioning.

Number of experiment	Arterial unsaturation percentage			Venous unsaturation percentage		
	A 1	A 2	A 3	V 1	V 2	V 3
P.O. 1	4·5	—	7·3	29·5	51·0	77·5
P.O. 2	7·0	24·5	8·8	38·6	73·6	36·8

A 1 = Arterial blood under normal conditions.
A 2 = Arterial blood after induction of pneumothorax.
A 3 = Arterial blood after induction of pneumothorax plus 5 minutes administration of oxygen.

V 1 = Venous blood under normal conditions.
V 2 = Venous blood after induction of pneumothorax.
V 3 = Venous blood after induction of pneumothorax plus 5 minutes administration of oxygen.

(*b*) After the bronchus was plugged oxygen was administered and the oxygen content of the blood estimated. Administration of oxygen in these experiments rapidly raised the arterial saturation to a point in excess of normal, the venous saturation returning also to a point above the first reading.

Number of experiment	Position of plug with reference to Rt. bronchus	Percentage arterial unsaturation			Percentage venous unsaturation			Duration of O_2 administration
		A 1	A 2	A 3	V 1	V 2	V 3	
B.O. 1	R. bronchus slightly blocked	8·8	50·9	36·9	26·3	82·4	61·4	5 mins.
B.O. 2	R. bronchus partially excluded	6·4	72·2	14·8	24·7	78·7	31·9	10 mins.
B.O. 3	R. bronchus clear	4·8	28·0	2·8	45·0	75·0	25·0	10 mins.

Note. In all these experiments L. bronchus completely blocked.
In experiments 1 and 2 R. bronchus partially overlapped also.
A 1, V 1, etc., as in previous table.

Conclusions.

The fall in the arterial and venous oxygen content produced by unilateral pneumothorax or occlusion of one bronchus is rapidly removed by administration of oxygen.

Anoxæmia and the administration of oxygen. By D. K. Adams and N. Morris. (*Preliminary communication.*)

III. *Effect of administration of oxygen previous to the induction of unilateral pneumothorax.*

Methods. As in last communication.

When oxygen was administered throughout the experiment there was no fall in the arterial oxygen saturation immediately after the induction of the pneumothorax. Ten minutes later, oxygen still being continuously administered the oxygen saturation had reached a higher figure than in normal blood.

No. of experiment	Arterial unsaturation percentage			Venous unsaturation percentage		
	A 1	A 2	A 3	V 1	V 2	V 3
1	26·0	9·9	2·0	52·0	58·0	43·0
2	5·0	5·0	—	29·0	—	—
3	11·0	15·0	11·0	59·0	46·0	55·0
4	11·0	4·0	3·5	21·5	22·0	—
5	8·5	6·5	6·5	22·5	25·5	56·5

A 1 and V 1 = Blood under normal conditions.
A 2 and V 2 = Blood immediately after induction of pneumothorax.
A 3 and V 3 = Blood 10 minutes later.

Two experiments were performed where the thorax was opened and artificial respiration carried on as well as the administration of oxygen. The right bronchus was then ligatured. We found that even with the administration of oxygen there was a gradual fall in the arterial saturation. In addition there was noticed a progressive failing of the cardiac musculature.

No. of experiment	Arterial unsaturation percentage		
	A 1	A 2	A 3
1	5·0	11·0	53·0
2	8·0	10·4	40·0

A 1 = Normal.
A 2 = Immediately after ligature of bronchus.
A 3 = 10 mins. later.

Conclusions.

Administration of oxygen previous to the induction of unilateral pneumothorax prevents a fall in the arterial saturation.

The progressive cardiac failure obtained after ligature of a bronchus may be a reflex phenomenon. This failing with the fall in arterial saturation is very suggestive of the condition of cyanosis found clinically in cases of heart-failure.

We desire to offer our best thanks to Prof. D. Noël Paton, F.R.S., LL.D., for helpful suggestions and criticism during the progress of the research.

The production of an electromotive force by the movement of salt solution past silver electrodes.

By W. E. L. Brown[1] and A. V. Hill.

The following observations were made during an attempt to detect a difference between the chlorine-ion concentrations of the plasma of reduced and oxidised blood.

Silver electrodes were constructed as shown in Fig. 1. *C* is a plate of silver, hammered from a globule made by fusing the end of a silver wire. This wire is joined to a copper wire at *D* by fusing in a blow-pipe flame, and the junction is embedded in paraffin wax in the tube *A*. This tube is sealed into the tube *B* with paraffin wax, and the whole is painted with black varnish between the lines *a*, in order to protect the silver plate from light. The electrodes were coated with silver chloride by making them the anode in a solution of sodium chloride, using a platinum cathode. The two electrodes were treated in the same way while they

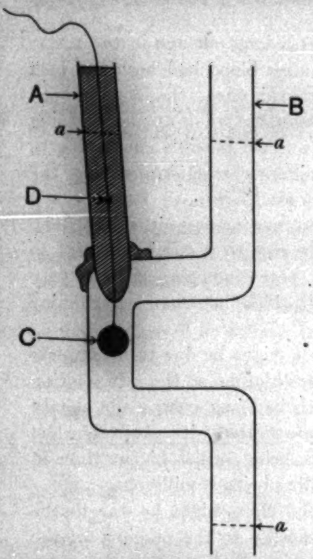

Fig. 1.

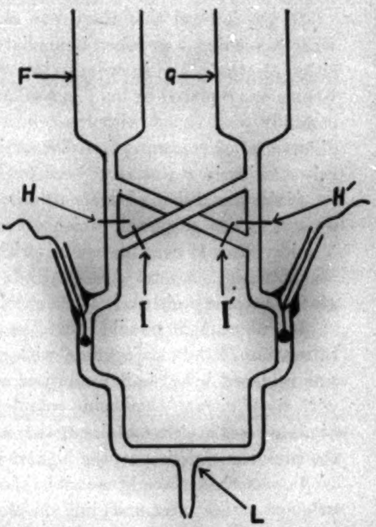

Fig. 2.

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[1] Working on behalf of the Medical Research Council.

were being made, and they were coated in series, so that their surfaces may be assumed to be similar.

The electrodes were set up as shown in Fig. 2: reduced blood was put in *F*, and oxidised blood in *G*. By releasing clips on the rubber pipes *H* and *H′*, it was possible to allow the two kinds of blood to run past the electrodes and out at the T-piece *L*. On closing the clips on *H* and *H′* and releasing those at *I* and *I′*, reduced blood was allowed to run past the electrode previously exposed to oxidised blood, and *vice versa*. Thus it was possible to eliminate effects due to differences between the two electrodes.

It was noticed that there was always a large change in the E.M.F. when a reading was taken immediately after blood had been run past either electrode, and in order to investigate this effect, the blood in both vessels was replaced by 0·9 p.c. NaCl solution, the whole apparatus being carefully washed out with the solution to avoid possible effects due to differences of concentration. To avoid differences of temperature, the salt solution in *F* was alone used for both electrodes.

It was found that while the solution was running past either electrode, an E.M.F. was produced, which gradually rose to a maximum of 4 to 5 millivolts. It was measured with a potentiometer, using a very sensitive galvanometer. The electrode past which the stream was running always became positive.

It was thought possible that the effect might be due to incomplete saturation of the moving fluid with silver chloride, so the salt solution was replaced by a similar solution, which had just been made cloudy with silver nitrate, thus being completely saturated with silver chloride. Similar results were obtained, the E.M.F. being rather higher than in the previous experiment, the highest reading being 8 millivolts.

It was then thought possible that the effect might be due to the velocity of the stream adding on algebraically to the mobility of the ions, in which case by reversing the velocity of the stream the effect would be reversed. To test this the connections of the apparatus were reversed, so that the T-piece was at the top of the electrodes, the exit tube being connected to the vessel *F*. The stream of salt solution now ran *from* the T-piece *to* the electrode. This arrangement gave about the same E.M.F. as the previous experiment had given, and the electrode past which the stream was running was still positively charged. Hence this explanation also fails.

The phenomenon seems therefore to occur at the electrode itself, and we can only suggest that it is due to a mechanical effect of the stream in (so to speak) washing away the chlorine ions of the electric double layer there, and so leaving the silver positively charged. Its existence indicates the necessity of avoiding movement of the fluid when using electrodes to determine ionic concentrations, and it may possibly be of interest in connection with manifestations of electricity in the body.

Since the above paper was read, the experiments have been repeated with uncoated electrodes of very pure silver (at least 999·9) given us by Mr C. T. Heycock, F.R.S. Similar results have been obtained, and the phenomenon is not due therefore to the use of impure silver.

The regulation of the supply of energy in muscular contraction.

By W. Hartree and A. V. Hill.

Experiments have been made at various temperatures on the relation between the duration of the stimulus and the total energy evolved in a maximal tetanic contraction, with the intention of determining the type of mechanism regulating the supply of energy in the stimulated muscle undergoing prolonged activity. The sartorius muscles of a frog were placed on the gold-nickel thermopile previously described and stimulated either by a single induction shock (representing zero duration of stimulus) or by the prolonged application, for any required duration, of an alternating current of 90 periods giving 180 stimuli per second. The heat was determined in absolute units and the amount of heat plotted against the duration of stimulus on a curve.

Various interesting relations were found. The production of heat was very rapid at first, a large amount being liberated by the single shock, and the rate of heat-production rapidly decreased and settled down within a fraction of a second to a steady rate depending on the temperature. The heat liberated by a single shock is considerably greater at a *lower* temperature, while the rate of heat production during the steady state finally attained is far greater at the *higher* temperature. All the curves cross at about 0·02 second duration of stimulus, so that for a stimulus of 0·02 second, change of temperature has little or no effect on the total heat production. The effect of temperature on the rate of heat production in the final steady state is exactly analogous to its effect on a chemical reaction, being of the exponential type and of the same order of magnitude, viz. increasing the rate 2·8 times for a rise of 10° C. The whole behaviour of the muscle liberating energy is analogous to that of a mechanical system in which an elastic bag is blown up through a narrow pipe with compressed air supplied from a reservoir. If the bag be kept closed the reservoir blows up the bag until an equilibrium is attained. On opening a valve in the bag the air stored in it rushes out in large amount at first and then at a rapidly decreasing rate, until a steady state is reached depending on the size of the pipe connecting the bag to the reservoir. We may suppose that the same type of mechanism exists in the muscle. The muscle has a store of lactic acid (exhibiting certain "elastic" properties as described below) kept up to its normal concentration by means of some chemical reaction corresponding to the supply pipe of the bag. On applying a single shock the permeability of the walls of the space in which the lactic acid is stored, rise, exhibiting the electric change, and during this rise of permeability the lactic acid is able to pass out and initiate the contraction, with the liberation of heat. As soon as some of the lactic acid has disappeared the chemical reaction supplying it gets to work, and if the stimulus be continued a steady state is rapidly attained in which the energy is being supplied at a rate determined only by the velocity of the chemical reaction providing the lactic acid.

The supply being maintained by a chemical reaction, it is natural that its velocity should have a temperature coefficient of the type described. According to this scheme the smaller production of heat resulting from a single shock at a higher temperature, is due to the fact that at a higher temperature the rise of permeability manifested by the electric change lasts for a shorter time and therefore less energy is able to escape. It is necessary to suppose that the system has some kind of elastic properties, otherwise it is not easy to see why the first shock is able to liberate so much more energy than the succeeding ones. We must assume therefore that the muscle contains some explosive or elastic system capable of being suddenly released. Such an elastic system is constituted chemically by a balanced reaction, where the products of the reaction are built up gradually, *e.g.* under the action of a ferment, until their concentration (analogous to the elastic back-pressure in the mechanical model) stops the action from progressing further. The sudden rise of permeability allows these products to disappear suddenly and the chemical reaction to begin moving forwards again. Thermodynamics is incapable of filling in the details of the picture, but it does seem capable in this way of giving a clue as to the general type of mechanism that the picture has to delineate.

Simple process for rendering permanent Golgi-Cox preparations[1].

By C. Da Fano.

In order to overcome the difficulty of cutting by means of the freezing microtome nervous tissues treated according to Cox's modification of Golgi's bichromate and sublimate method, Sanders[2], Bremer[3] and others have suggested washing pieces in many changes of alcohol and imbedding them in celloidin, this with the object also of rendering preparations more permanent by removing the excess of corrosive sublimate not utilised by the reaction, and which still permeates the tissues. As a matter of fact sections of pieces thus treated are very easily cut and transferred from one to another fluid without danger of injuring them. Moreover, they can be counterstained and the impregnation keeps well, particularly if sections are mounted without a cover glass. But in such preparations, sometimes quickly, sometimes slowly, opaque granules and minute needle-like crystals become almost always developed which are very undesirable and end by rendering preparations more or less unserviceable.

To avoid this I find that sections can be successfully treated much in the same way as by the so-called process of toning and fixing Bielschowsky preparations and the like, as already communicated in a former note[4]. I proceed thus: pieces, which by a trial section have been found well impregnated, are washed for some hours in distilled water and then brought, through many changes of alcohol of ascending strength, into absolute alcohol and then imbedded in celloidin in the usual way. The celloidin blocks are hardened in 70 p.c. alcohol where they can be safely left for many days or weeks. Sections of the desired thickness are collected in 60 p.c. alcohol, transferred into distilled water and here thoroughly washed. They are then treated for 5–10 minutes with 5 p.c. ammonia and washed over again in two or three changes of distilled water. At this point toning is carried out by means of the usual slightly acidified 0·2 p.c. gold chloride solution in which sections are left for 10–15–20 minutes according to thickness. After quick washing in distilled water they are passed for 3–5 minutes into 5 p.c. sodium hyposulphite and washed once more in distilled water. From this they are passed successively into 30, 50, 70 p.c. alcohols to each of which one drop of saturated iodine tincture to every 5 c.c. of alcohol has been added. Sections remain in each alcohol 10–15 minutes and are lastly transferred into pure 70 p.c. alcohol.

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[1] The expenses of the investigation were in part defrayed by a Government Grant from the Royal Society.

[2] Sanders (*in litt.*) *see* A. B. Lee. *Vade Mecum.* 1913 ed.

[3] Bremer. *Anat. Rec.* 4. p. 263. 1910.

[4] Da Fano. *Proc. Physiol. Soc.* 53. p. xcii. 1920.

At this point the process is ended and one can proceed to mount sections in the usual way or re-transfer them into distilled water, counterstain them slightly with a carmine solution, dehydrate them with alcohol of ascending strength up to 95 p.c., pass them through two changes of carbol-xylol and mount them under a thin cover-glass in xylol-colophonium or balsam. If desirable and safe, the celloidin can be removed before definite mounting by passing sections through absolute alcohol, and alcohol-ether if necessary.

The process is simpler than the platinum substitutions of Robertson and MacDonald[1], and is so easily carried through that many sections can be manipulated at the same time. It should therefore become useful for preparing beforehand sections for class purposes. It must, however, be pointed out that the process does not give satisfactory results by Golgi's chromate of silver method. I cannot at present state if it could be used for rendering permanent sections of material treated by Golgi's bichromate and sublimate method, and I am still investigating if the toning-and-fixing process proposed by Golgi[2] for such sections could, with some slight modification, be usefully employed for Golgi-Cox specimens.

Preparations from cases of epidemic (lethargic) encephalitis[3].

By C. Da Fano.

In a preliminary note on the histopathology of epidemic (lethargic) encephalitis[4] a brief description was given by me of certain minute bodies seen within and without nerve-cells of various regions of the central nervous system in different cases of the disease.

The histological methods used were simple. All preparations of nervous tissue were made from material fixed in 95 p.c. alcohol and imbedded in celloidin. Sections were stained either with 1 p.c. toluidine blue or with polychrome methylene blue according to the classical rules of Nissl's method, and then mounted in benzol-colophonium. The sections were, however, carefully freed from all celloidin immediately

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[1] Robertson and MacDonald. *Journ. Ment. Sc.* 47. p. 327. 1901.

[2] Golgi. *Opera Omnia*, 2. p. 607. 1903.

[3] Towards the expenses of this research a grant was made by the British Medical Association.

[4] Da Fano. *Brit. Med. Journ.* i. 1921.

after cutting, and the staining was repeated a second and a third time after having each time completely differentiated the sections with 96 p.c. alcohol. This repetition of staining and differentiating, particularly when carried out with relatively old solutions of toluidine blue, has, in my opinion, a double advantage. In the first instance it greatly helps in imparting to the cytoplasm of nerve-cells, neuroglia-cells and other elements of the preparations a more or less intense purple colour, on which differently stained parts such as nuclei, granules and the like stand out sharply and definitely. In the second place the repeated staining appears to render the specimens more resistant to the injurious influence of time. Indeed, some of those I now demonstrate were prepared over two years ago and their colour seems to me to have faded very little.

As to the peculiar forms shown in my preparations, in consideration of the fact that a description of them has already been given elsewhere, it seems sufficient to point out here that they consist of very small granules generally situated in the deep as well as in the superficial parts of the bodies of nerve-cells, but sometimes found also outside them in their immediate vicinity. The granules are easily distinguished because of their blue stain contrasting with the purple of the fundamental cytoplasmic framework and because of minute light areas which surround nearly all of them. These areas look, under moderate magnification, like halos, but under high power they appear as very delicate, though sufficiently well-defined, bodies whitish or slightly bluish in colour and irregularly round or oval in form. As a rule there is one granule in each body, but in some of them two granules, arranged like diplococci, can be seen. Most of the bodies are discrete and only a few appear united in dumb-bell shaped forms. For these peculiar appearances the term "minute bodies" has been *pro tempore* proposed comprising in the term also the granules which seem to occupy their centre.

The "bodies" are always found in more or less altered cells, but their occurrence in a pure form is rather rare, and certainly confined to specimens from acute cases of the disease. In most instances they are seen together with a variable quantity of granular pigment-like material which is stained in various shades of green by toluidine and polychrome methylene blue. I am still investigating the relation which may possibly exist between the "bodies" and this pigment-like material, the occurrence of which in places where brown or black pigment is not generally found has already been pointed out since my first communication on the subject published in collaboration with H. Ingleby[1].

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[1] Da Fano and Ingleby. *Proc. Roy. Soc. Med.* **12.** p. 42. 1919.

It may be of interest to add that "bodies" similar in shape, structure and staining properties have been found by me also within and without the cells infiltrating a salivary gland from an acute case of the disease.

No definite opinion has been at present expressed about the possible significance of such findings, and only the suggestion has tentatively been put forward that they may be related to the presence in the tissues of a living agent, the cause of the disease.

The solution of casein by sodium carbonate—an example of reversible coagulation. By J. MELLANBY.

The bicarbonate hypothesis for the transport of CO_2 in blood assumes that proteins can act as acids capable of decomposing sodium carbonate. To test the general validity of this assumption the conditions under which two proteins (casein and acidic caseinogen) dissolve in the presence of carbonates were determined. Casein was prepared by adding a minimal quantity of neutral gastric rennin to separated milk. The precipitate was washed with water until an approximately pure suspension of casein was obtained. The casein was not dissolved on adding solid calcium carbonate to the suspension, but solution was readily produced by ·2 p.c. Na_2CO_3. During the solution of casein in ·2 p.c. Na_2CO_3, air, free from CO_2, was pulled through the liquid and passed into baryta water. There was no evidence of the evolution of CO_2 during the solution of the casein in the sodium carbonate. It is evident, therefore, that the solution of casein in sodium carbonate cannot be represented by the equation:

$$\text{Casein} + Na_2CO_3 \rightarrow \text{Na caseinate} + CO_2.$$

The solution of casein in sodium carbonate affords an interesting example of reversible coagulation. A typical coagulum comparable to that produced in milk by rennin can be made by adding a trace of calcium chloride to the casein solution. This phenomenon can be explained on the assumption that during the coagulation of milk by rennin the emulsoid colloid (caseinogen) is changed into the suspensoid colloid (paracasein) and this suspensoid colloid is precipitated from solution by the divalent calcium ions in milk. The precipitation of paracasein by calcium involves an adsorptive union of the protein and calcium salt. The resolution of casein in sodium carbonate is effected by the removal of the calcium ions from the paracasein particles, and recoagulation is obtained by adding an excess of calcium salt to the re-dissolved paracasein.

The meaning of records made with the hot wire sphygmograph.

By A. V. Hill.

Investigations have been continued with the hot wire sphygmograph, and a more satisfactory instrument has been designed employing 11 μ platinum wire made by Messrs Johnson and Matthey. The advantage of platinum wire is that it does not burn out as did the copper wire previously used, and it appears also to be more rapid in its action. Ample sensitivity is obtained with the wire just below red heat, employing the galvanometer string as tight as it can safely be made. Since my previous communication the galvanometer has been made more sensitive and its movements are now dead beat. The type of record obtained with the latest instrument is shown in Fig. 1; it will be seen that the two main features are (i) the first large wave corresponding to the rush of blood into the aorta on the opening of the valves, and (ii) the second smaller wave due to the sudden fall of pressure at and after the end of systole, culminating exactly at the moment the valves close. The beginning of the first curve therefore represents the opening of the valves and the sharp peak on the second curve represents their closure. This is well shown by the simultaneous optical record shown in white on Fig. 1, which was taken on the same plate by means of a small tambour placed in series with the tube containing the hot wire. A mirror placed excentrically on the

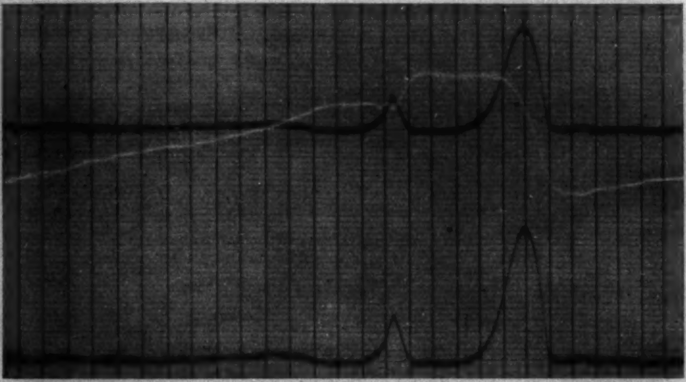


Fig. 1. Black curves hot-wire records of two similar instruments in series. White curve mechanical record made with tambour. Carotid artery. Curves read from right to left. Time in 1/25 seconds.

tambour reflected a spot of light on to the plate and the movements of this spot of light represent the changes of volume of the artery. It is seen that the hot wire record rises at the moment when the artery begins to swell, and that the second peak of the hot wire record occurs exactly at the same moment as the dicrotic notch. The hot wire sphygmograph gives no direct evidence as to the absolute value of the pressure or of the movements of the artery, and its usefulness depends upon the quickness with which it is capable of acting. It may be regarded as a means of recording the moment at which the various waves produced in the arterial system occur at any required point. For example it will measure with accuracy the time during which the valves of the heart are open. Moreover by employing a double fibre case in the string galvanometer and two hot wire instruments it is possible to record simultaneously the pulse at two different parts of the body and therefore to find the velocity of the pulse wave under various conditions. Fig. 2 shows simultaneous observations of the right carotid artery and the right radial artery on the same plate, and the interval between these two records gives the velocity of the pulse wave which in this case works out to 7·5 metres per sec. In order to be sure that the two hot wire instruments are exactly similar and record the same thing at identically the same moment they may be put in series with one another on the same pipe, leading say

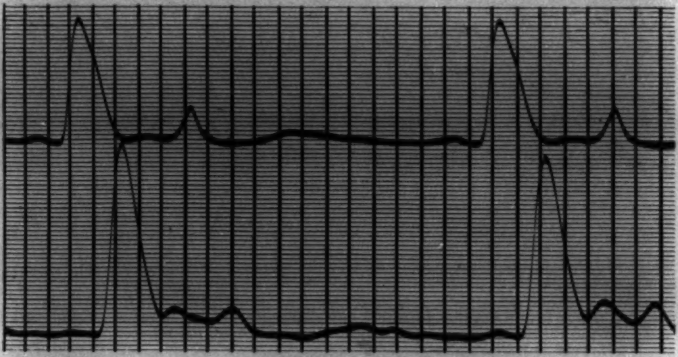


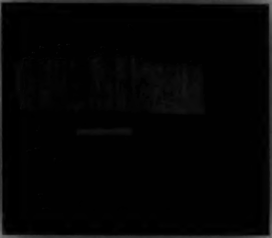
Fig. 2. Upper curve hot wire record of right carotid artery. Lower curve hot wire record of right radial artery. The interval between the two records is about 0·067 second. This corresponds, in the subject employed, to 50 cm. travel of the pulse, viz. 61 cm. from the sterno-clavicular joint to the radial artery, less 11 cm. from the sterno-clavicular joint to the carotid artery; the pulse therefore goes 7·5 metres per second. Curves read from left to right. Time in 1/25 seconds.

from the carotid artery. The two electric records in Fig. 1 were made in this way and it is seen that the two instruments are giving exactly similar curves; it is justifiable therefore to assume that the time interval as measured in Fig. 2 really represents the interval required for the transmission of the pulse wave.

The effect of i-inositol on the isolated heart of the frog. By J. A. HEWITT and D. DE SOUZA.

Perfusion of the frog's heart was carried out by Symes's method, an improved cannula devised by Dr Locke being used instead of the ordinary one. The heart was perfused at a pressure of 3–4 cm. of water. The concentration of inositol employed varied from 1/11,500 to 1/750 in Ringer's solution. ($NaCl$ 0·6 p.c., KCl 0·026 p.c., $CaCl_2 . 6H_2O$ 0·032 p.c.)

The usual result obtained is shown in the typical tracing reproduced.



The heart rapidly responds to the inositol by increasing the force of its beat, and just as rapidly returns to its original condition when the inositol is replaced by Ringer's solution alone. This result is obtained with fresh hearts and with hearts that have been previously perfused with Ringer's solution for any period up to six hours.

In six out of 25 experiments inositol had no apparent effect on the heart, and in two its perfusion led to a diminution in the force of the beat. For this exceptional result we have at present no explanation to offer.

The rate of the heart is, as a rule, not affected by inositol.

The stimulating effect is clearly on the muscle. It occurs in the atropinised heart. Moreover, a concentration of 6 p.c., such as was employed by Sachs(1), is strongly toxic and causes rapid contracture of the ventricle and the systolic stand-still obtained by him.

(1) Sachs. *Arch. f. d. ges. Physiol.* 115. p. 550. 1906.

The decomposition of carbonates by acidic caseinogen. By J. MELLANBY.

Caseinogen, the main protein of milk, is readily soluble in water forming a milk-white solution. Acidic caseinogen may be precipitated from a solution of it by the addition of acid. Acidic caseinogen is insoluble in water, but dissolves when added to water in which carbonates are suspended or dissolved. During the process of solution CO_2 is evolved.

I have previously shown that caseinogen consists of a complex of protein and calcium phosphate, approximately 3600 grams of protein being associated with a gram molecule of calcium phosphate. On adding acid to a solution of caseinogen it may be observed (1) that the amount of acid required to precipitate the protein is approximately equal to the amount of calcium phosphate in the protein complex, (2) precipitation is suddenly produced when acid is added in increasing quantities to a solution of caseinogen, and (3) the filtrate obtained after the precipitation of acidic caseinogen contains only a trace of acid. These facts indicate that the precipitation of acidic caseinogen depends upon the adsorption of the added acid by the caseinogen, and that the solution of acidic caseinogen by carbonates is due to the neutralisation of the adsorbed acid. In fact, that the evolution of CO_2 on dissolving acidic caseinogen by carbonates is not due to the protein but to the acid adsorbed by the protein.

To test this hypothesis acidic caseinogen was made by precipitation with phosphoric acid and the amount of phosphorus contained in the precipitate was determined after washing for varying periods of time. After one washing the precipitated protein contained a quantity of phosphorus equal to that in the added acid; after ten washings the quantity of phosphorus fell to that contained in acidic caseinogen prepared by means of acetic acid. It appears, therefore, that on the precipitation of acidic caseinogen, the added acid first associates with the protein, but that the complex is unstable and by continuous washing the acid may be removed from the precipitate. Acidic caseinogen was also precipitated from milk by hydrochloric acid and the precipitate was washed until the filtrates were free from chloride. The protein suspension was dried and ashed, a little KOH having been previously added to form a non-volatile neutral salt with any HCl which might have been associated with the protein. The ash contained no chloride. It is evident, therefore, that acidic caseinogen if adequately washed contains no trace of precipitating acid in its complex.

Pure acidic caseingoen slowly reddens blue litmus paper and readily dissolves when calcium carbonate or sodium carbonate is added to a suspension of it in water. During solution CO_2 is evolved. Approximately 2200 grams of acidic caseinogen liberate a gram molecular weight of CO_2 when dissolved in calcium carbonate. This value agrees with those obtained by other observers for the value of the combining equivalent of acidic caseinogen from a consideration of the constitution of calcium caseinogenates (Lacqueur and Sackur 1135; Robertson 1250; Bosworth and Van Slyke 1111).

Acidic caseinogen was dissolved in water containing calcium carbonate in suspension. The resulting solution was centrifuged for 30 mins. to free it from undissolved calcium carbonate. The solution evolved no CO_2 when submitted to the action of sulphuric acid in a vacuum, but after being exposed to alveolar air for 30 mins. it contained 18 p.c. CO_2.

The experimental results indicate (1) that proteins can function as acids capable of decomposing carbonates, and (2) that these salts of protein can carry CO_2.

The chlorine ion concentration of plasma of oxidised and reduced blood. By W. E. L. Brown[1] and A. V. Hill.

L. J. Henderson has shown that the chlorine concentration of plasma of reduced blood is slightly less than that of the same blood after shaking with oxygen. If this be the case, we should expect that the chlorine ion concentration would be less also, as it is unlikely that the chlorine coming from the inside of the corpuscle would appear outside in a combined form. We have attempted therefore to determine the difference in chlorine ion concentration by a direct electrometric method. A quantity of blood was reduced by boiling in a vacuum, saturated with AgCl, and half of it shaken with oxygen. The two halves were placed separately in two cylinders connected by rubber pipes provided with clips, to two electrode tubes joined together by a T-piece. The electrodes were of silver chloride formed electrically on the surface of small silver plates. In order to prevent any action of light on the chloride the tubes in which the electrodes were mounted were painted with a thick coat of black varnish. Reduced blood was run past one electrode and oxidised blood past the other electrode, the two different forms of blood meeting in the T-piece.

[1] Working on behalf of the Medical Research Council.

The electrodes were then connected to a potentiometer, and a very sensitive galvanometer used to determine the balance. It was found necessary to wait for a minute or two after running the solution past either electrode for reasons explained in another communication. After an experiment had been made with oxidised blood on one electrode and reduced blood on the other electrode the system was changed over, reduced blood being placed on the first electrode and oxidised blood on the second. Half the difference between the two E.M.F.'s observed was taken as the true E.M.F. caused by the supposed difference of Cl-ion concentration. Reading to the nearest 0·02 millivolt, and calibrating the apparatus with known concentrations of NaCl before and after the observations, it appeared that the concentration difference was very small, too small to be certainly determined by the means available. The difference observed by Henderson was also very small, and apparently below the limit of possible observation by an electrometric method.

Rapid reexcitation in the mammalian auricle. By A. N. Drury and T. Lewis[1]. (*Preliminary notice.*)

In testing the refractory period of the mammalian auricle under vagal stimulation, it has been found that the auricle is easily thrown into a state which we term "rapid reexcitation." It is a state in which the excitation waves succeed each other at rates of from 1500 to 3000 per minute, and which persists so long as the heart remains under inhibition and despite the withdrawal of the shocks which have provoked it. The rapid excitation waves are not local, but spread as single waves over the whole exposed surface of the auricle. It may be said, therefore, that the auricle as a whole is beating (and almost co-ordinately) at rates of 1500 to 3000 per minute. The ability of the muscle to respond at these rates is consequent upon the great reduction of the refractory period, induced by vagal stimulation; for under vagal stimulation the R.P. falls to 0·02 or 0·03 of a second. These values agree remarkably with the rates prevailing in the state of rapid reexcitation, in which the lengths of the cycles lie between 0·02 and 0·04 of a second.

Rapid reexcitation is regarded as being a circus movement, comparable to that prevailing in flutter, though on a diminutive scale.

As has been shown by many observers, it is a condition frequently provoked in fluttering or fibrillating auricles by stimulating the vagus. It may also be produced in an auricle responding to rhythmic shocks under vagal stimulation, if a single additional shock enters the muscle. If this single shock enters at a critical period of the cycle, namely, from 0·025 of a second (the end of the R.P.) up to about 0·07 of a second after the last rhythmic shock, the reaction is invariable; it is not produced if the single shock falls outside these time limits.

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[1] Working on behalf of the Medical Research Council.

Likewise rapid reexcitation is provoked if, under vagal stimulation, two break shocks enter the muscle, providing that these two shocks have a time interval of from about 0·025 to 0·07 of a second between them.

Although the reason why this period is critical is being submitted to further investigation, we believe that it is critical because the muscle is in a partially refractory state, and that reentry of the first excitation wave depends upon this state of partial refractoriness.

The regulation of the water content of the human organism.

By E. F. ADOLPH.

The addition of water to the body was attempted in several ways. Ingesting water by mouth led to the complete excretion of the whole quantity through the kidneys. Thus when 2750 c.c. was drunk in 5 hours, 2900 c.c. had been excreted by the end of 7 hours. If the water was taken faster than the kidneys could excrete it, what was absorbed into the body was kept only until the kidneys were able to handle it. The ingestion of isotonic salt solution gave a very mild diuresis of 100 c.c. an hour, which lasted two days when two litres of solution were drunk; the augmented body weight gradually diminishing to its normal level. Hypertonic solutions of urea, NaCl, or other salts led to a rapid diuresis which effected an almost immediate elimination of the water, and in addition induced the loss of water from the tissues. When salt was taken, followed in two hours by a large amount of water, the diuresis initiated by the salt did not stop, but was augmented so as to quicken the elimination of salt. When water was drunk, followed by salt, the water diuresis was inhibited very sharply, and the retained fluid behaved like isotonic salt solution. In general isotonic salt solutions are retained longer than solutions containing other proportions of water and salt.

The reduction of the body's water content was effected in several experiments by stopping the water intake, by exercising, by sweating, by hot bathing, by inducing diarrhœa, and by inducing diuresis. The first method is most preferable because the water content was depleted in that case without lowering the reserves of salts and nutrients, which were kept up by eating concentrated foods. In each experiment the body weight fell gradually, and was completely restored by drinking enough water (3 to 4 kilos) to bring the body weight to its previous level. Further water ingestion induced immediate diuresis. If during dehydration salt or urea was taken, diuresis resulted, but in a manner more economical of water than when the body had its full quota of water. Not only was the urinary concentration of the diuretic substance raised, but its elimination was spread over a longer period, indicating that water was less readily and less quickly mobilized.

The detection and measurement of the state of the body's water content may be fairly exact. The sensation of thirst is very sensitive to sudden changes in the water percentage in the body, but may become inured to prolonged dehydration. Blood concentration and dilution are measurable, and indicate that variations in water content are distributed throughout the body, but unequally. Thus, a dehydration of 5 p.c. of the weight increased the blood's hæmoglobin concentration 10 p.c. A third measure of tissue hydration is kidney activity. Under uniform conditions the output of urine is exceedingly constant, and in the subject of these experiments is about 45 c.c. an hour when sitting at rest. In extreme dehydration on full diet the urine rate may drop as low as 17 c.c. an hour.

The most delicate index of the water reserve is the experimental production of diuresis by salts or urea. The diuresis lasts longer in dehydration, ends very gradually, and eliminates only about two-thirds as much of the diuretic substance in 12 hours as when the organism has plenty of water. The highest rate of water excretion when dehydrated is much less, and is attained more quickly. The maximal concentration of the diuretic substance is greater, and is attained more slowly. In the case of chloride diuresis there is no compensatory retention of water subsequent to the completion of diuresis, as there is when the body is hydrated. At no stage of dehydration does diuresis fail to be produced by salts or urea.

On the substance responsible for capillary tonus. By A. Krogh and G. A. Harrop. (*Preliminary communication.*)

In the web of the frog (*R. temporaria*) the capillaries are normally open, but so far contracted that the corpuscles can pass through only one by one and in most places with some deformation. When the femoral artery is clipped for 20 minutes and then opened again the capillaries are very strongly dilated, but usually they will regain their normal tone in 10–15 minutes. Some substance in the blood must be responsible for this action, and to find it we have begun a series of perfusion experiments through the femoral artery of frogs.

We find, as was to be expected, that in frogs, Ringer cannot maintain the tonus of capillaries and that the addition of gum or washed mammalian corpuscles does not make any difference. By perfusion with defibrinated and slightly diluted ox blood, however, the tonus can be maintained or recovered after dilatation. Ox serum has the same property, and when 200 c.c. of Ringer solution are dialysed against 1 l. ox blood for 12 hours the dialysate contains the active principle which does not become inactivated by a brief heating to the boiling point.

Experiments to isolate and determine this hitherto unknown substance are in progress.

Some observations on stasis and œdema. By A. Krogh and G. A. Harrop. (*Preliminary communication.*)

When a rapid and considerable dilatation of capillaries in the frog's tongue is brought about by any suitable kind of stimulation, directly or indirectly through nerves, it very often happens that the dilated capillaries in a few minutes or less become completely packed with red corpuscles, while the plasma apparently disappears and the blood flow stops. The same degree of dilatation does not generally lead to stasis, when brought about slowly and cautiously. The capillary pressure has very little influence upon the result, since the stasis develops quite as easily after urethane, which leaves the arterioles contracted, as after mechanical stimulation acting through the dilator nerve fibres, which will dilate the arterioles along with the capillaries and produce a rapid flow and high pressure.

The explanation of the stasis which suggests itself is that the rapid dilatation gives rise to small openings between the endothelial cells through which the plasma can pass out. In order to test this conception and obtain some idea about the size of the openings we have made the following experiments.

In urethanised frogs (*R. esculenta*) the ventral surface of the tongue was spread and a suspension of india ink particles (Pelikan Perl Tusch, Günther-Wagner) run into the cutaneous vein. When the india ink became visible in the vessels of the tongue a group of capillaries were caused to dilate by means of a drop of 25 p.c. urethane. Though stasis developed rapidly the india ink remained within the vessels except in one experiment at a single point.

The experiment was repeated with a solution of the colloidal dye vital red and with soluble starch. Both these substances penetrate easily through the wall of rapidly dilated capillaries though they are quantitatively retained in normal vessels. The experiment with vital red is very beautiful. Just after the dilatation of a capillary a narrow red zone is seen to surround it which later, when stasis has developed, fades slowly by diffusion into the surrounding tissue.

The experiments will be continued, if possible, with metal sols containing particles of well defined size.

PROCEEDINGS

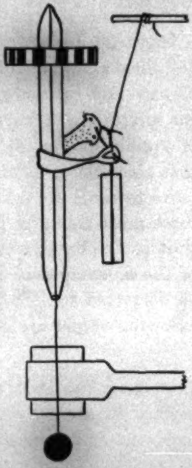
OF THE

PHYSIOLOGICAL SOCIETY,

February 12, 1921.

A simple apparatus to demonstrate activity of cilia. BY O. INCHLEY.

A glass tube 10 cm. long and about 5 to 8 mm. in diameter, is tapered down and sealed at one end, the other tapered end being left open and large enough to admit a vertically held hatpin which supports it (see Fig.). A strip of frog's ciliated membrane is looped round this, the ciliated surface being in contact with it; the glass spindle will be slowly rotated by the activity of the cilia. Near its upper end the spindle carries a disc of cork, about 3 cm. in diameter and graduated at the circumference to render the rotation obvious to the class.



The loop of membrane is held together at its extremities upon a suitably bent pin, heavily weighted, and supported by a thread. The tension on the membrane is easily regulated by altering the inclination of this thread, by moving outwards its fixed support above.

A drip feed of frog's oxygenated Ringer is arranged to play upon the glass spindle.

For uniform motion the glass and cork must be well balanced on the needle; this can be attained by holding the needle inclined at 45° and rubbing off the heavier side of the cork until the spindle will stop dead in any position indifferently.

Any slowing of the movement during the first hour is almost certainly due to ropes of mucus tying the spindle; this can be removed by a camel-hair brush applied to both membrane and spindle.

The strip of membrane is obtained from the pithed frog by dissecting out the roof of the mouth, pharynx, and œsophagus, together with the tip of the nose and a piece of stomach to be impaled by the supporting pin.

Another form of apparatus may be used with the membrane immersed in oxygenated Ringer: here the supporting pin is held vertically downwards, and the glass tube and cork is floated up against it. In this case the balancing must be done by inclining the needle under water, the process being similar to that described above.

Shift of the absorption spectra of oxy- and carbon-monoxide-hæmoglobin with change of temperature. By H. HARTRIDGE.

Recent experiments with my reversion spectroscope have shown that the absorption spectra of both oxy- and carbon-monoxide-hæmoglobin shift towards the red with rise of temperature. The movement per degree is about ·28 Angstrom unit for the *α* bands of both compounds. I have described a similar shift with rise of temperature of the bands of mixtures of oxy- and carbon-monoxide-hæmoglobin in equilibrium with suitable gas mixtures and I erroneously ascribed this to a change in the position of equilibrium due to change in temperature. It is now clear, however, that since the *α* bands of both oxy- and carbon-monoxide-hæmoglobin move towards the red with rise of temperature the bands of mixtures of these must similarly move and this is therefore the explanation of the shift in the bands that I had observed. If the percentage saturations of the six samples of dilute blood, in equilibrium with the gas mixtures, be corrected for this shift of the bands with rise of temperature the following values are obtained:

	Percentage saturation with CO					
Temp.	Sample (1)	Sample (2)	Sample (3)	Sample (4)	Sample (5)	Sample (6)
0	18·5	26	44·5	59	66	69·5
10	24	32·5	45·5	61·5	66·6	70·5
20	26	35	48	59	68·8	72
30	29	39	47·5	59·5	69	72
40	32	39	47	56	66·9	72
50	—	42·6	48·5	57·5	64·5	71·2
60	—	—	49·5	58	63·0	71

It will be seen from the above values that the percentage saturation of blood with CO gas rises with rise of temperature at low concentrations, does not appreciably change at medium concentrations, and falls slightly at high concentrations. These changes have been verified by a direct series of measurements as follows: To defibrinated sheep's blood was added an equal quantity of water so as to lake the corpuscles. A few c.c. of this were placed into a tonometer containing a suitable mixture of coal gas and air, so that when equilibrium was finally established the blood should be partially saturated with CO gas. After roughly half an hour's rotation the blood was withdrawn from the tonometer; some of it was then placed into two small test-tubes.

These test-tubes were fitted with rubber corks through each of which passed two glass tubes, one nearly reaching the bottom, the other just entering. These were now connected with the tonometer so that the gas displaced by admitting water should pass over the surfaces of the samples of blood in the test-tubes. The test-tubes were now mounted on a shaking machine so that one was surrounded by cold water, the other by hot, gas from the tonometer being arranged to pass at will through the tubes which were being shaken. After half an hour the samples were caused to reach room temperature and were then diluted with water and examined with the reversion spectroscope.

The following values were obtained:

Hot (about 40° C.)	Cold (about 8° C.)	Difference
26·8	16·2	+10·6
41·1	27·2	+13·9
43·0	32·5	+10·5
42·25	38·05	+ 4·2
46·1	41·45	+ 4·65
59·1	56·6	+ 2·5
61·5	59·5	+ 2·0
62·2	62·2	± 0
61·85	65·8	− 3·95
62·5	66	− 3·5
63·45	65·05	− 1·6
67·3	69·15	− 1·85
67·4	69·15	− 1·75
70·95	73·4	− 2·55
79·65	80·6	− ·95
80·0	82·9	− 2·90

The above figures agree with those previously obtained in showing that at low saturation the values rise with rise of temperature, at medium saturation they remain approximately constant, whereas at high concentration they fall slightly with rise of temperature.

The above conclusion is not in precise agreement with those of either Haldane and Lorraine Smith or Douglas and Haldane. The former experimenters found little or no change, the latter a fall of saturation with rise of temperature.

Since writing the above I have measured the positions of the bands of oxy-hæmoglobin at the temperature of liquid air. For the purpose of this experiment a film of hæmoglobin in gelatin was used, since an aqueous solution when frozen was found to be too opaque for spectroscopic examination. The bands at this temperature look much sharper to the eye than those at laboratory temperature, and are shifted towards the violet approximately 41 A.U., *i.e.* a shift per degree of ·205 A.U.

With regard to the cause of this band shift it cannot be due to change in the refractive index of the solvent (in the above case water R.I. = 1·333) with change of temperature, because a solution of hæmoglobin in glycerine (R.I. = 1·47) has absorption bands that are almost exactly in the same positions as those of the aqueous solution, it would seem that some intra-molecular change is responsible for the band shift.

The influence of ventilation on massive infection. By S. R. Douglas and Leonard Hill.

In a room measuring 20 × 16 × 13½ ft. with two steam radiators turned on and windows and door closed the dry bulb temperature was 21·4° C. and the wet bulb 15·5° C., the dry kata-thermometer cooling power 4·3. The room felt over-warm and stuffy. This room was sprayed with 7·5 c.c. of a dilute culture of a coliform bacillus (from the rabbit), the spray being blown into the current from a blower which dispersed it towards the ceiling. The dilute culture fluid contained 300–500 million bacilli per c.c.

Petri dishes, containing Holt, Harris and Teague's medium, were exposed for 2 minutes at five places at table height, immediately after the spraying, and at 5, 10, 16 and 20 minutes afterwards.

The experiment was repeated with the windows so far opened as to give a fresh and comfortable feeling to the atmosphere.

The dry bulb was in this case 17·2° C., the wet bulb 12·8° C., the dry kata-thermometer cooling power 6·0. The Petri plates after incubation gave the following average counts:

	Stuffy room		Well ventilated room	
Exposure	Average no. of colonies	Total area of plates	Average no. of colonies	Total area of plates
1	1468	266 sq. cm.	1593	250 sq. cm.
2	634	263	446	250
3	272	272	54	260
4	118	263	7	314
5	76	272	1	250

This experiment, confirmed by others, shows that ventilation by open windows has a most marked effect in lessening massive infection, *e.g.* from saliva spray coughed, sneezed or spoken into a room.

The action of anæsthetics on the respiratory centre. By J. W. TREVAN and E. BOOCK.

Cushny (*Journ. Pharm. and Exp. Ther.* VI. 1914, p. 451) showed that CO_2 did not produce hyperpnœa in very deeply anæsthetised animals. We have investigated the action of CO_2 by a different method. Cats were used. Respiration was recorded by allowing the animals to breathe through a tracheal canula into a closed bottle connected with a bellows recorder. CO_2 was absorbed by soda in the bottle, and O_2 was added at a rate sufficient to compensate for the oxygen consumption of the animal. The glass tracheal canula had a branch in which a rubber stopper was fixed. Through the stopper a fine tube passed down to the bifurcation of the trachea. By means of this tube samples of alveolar air were obtained. The plasma bicarbonate was determined, by v. Slyke's method, in the blood as drawn from an artery. Variations in the alveolar CO_2 were brought about by varying the dead space of the apparatus, by connecting the tracheal canula with the bottle by tubes of wide bore and varying length. The reaction of the blood was estimated by a modification of Hasselbalch's formula applied to the plasma. The figures given correspond to the figures usually accepted as correct on the basis of *H*-electrode measurements. Lovatt Evans' results[1] suggest that they may have to be multiplied by 0·63. But this uncertainty as to the absolute value of the *H*-ion concentration does not affect the conclusions drawn, since all the values are affected proportionately.

The ventilation at different alveolar CO_2 tensions was measured by the rate and extent of the movements of the bellows recorder, first in Sherrington decerebrate cats which had recovered from the anæsthetic, then in both decerebrate and whole cats subjected to the action of large doses of urethane (up to 6 grams per kilo.). In decerebrated cats the onset of anæsthesia after the injection of urethane was shown by the disappearance of decerebrate rigidity, the reflexes persisting. At this stage there was no alteration in the response to CO_2, so that the assumption was made that the condition of the respiratory centre in whole cats, in whom the same anæsthesia had not led to disappearance of the corneal reflex, could be taken as normal. The subsequent course of the effect on the respiratory centre in both decerebrate and normal cats was the same, and is detailed below.

The ventilation at different alveolar CO_2 tensions was plotted against the calculated *H*-ion concentration. The points lie along a straight line intersecting the base line between 0·24 and 0·29 × λ 10⁻⁷ in different cats. This point represents the apnœic threshold, as can be shown in decerebrate animals by determining the reaction of the blood at a forced ventilation which is just sufficient to produce apnœa. There are three stages in the effect of urethane on the response to reaction changes.

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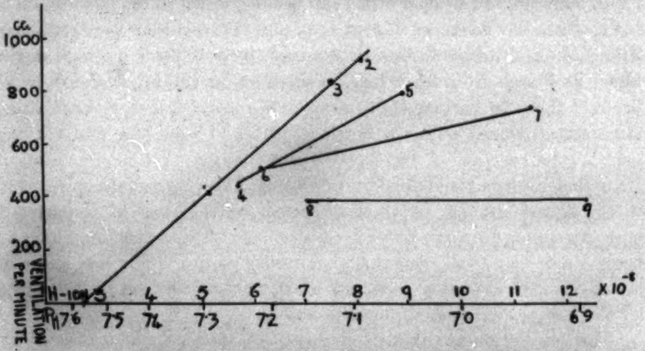
[1] *Proc. Phys. Soc.* Dec. 19, 1920.

1. As the reflexes disappear, the slope of the graph connecting the ventilation with the reaction of the blood is diminished, that is the sensitivity of the centre is diminished; the apnœic threshold is unchanged.

2. Later the slope of the graph diminishes further, but it intersects the base line on the alkaline side of the original apnœic threshold.



Decerebrate cat anæsthetised by the injection of urethane subcutaneously five hours after decerebration (Sherrington's method). Ventilation plotted against reaction of plasma. Observations numbered in the order in which they were made. The straight lines represent the sensitivity of the centre at successive intervals of about 35 minutes each.

3. The ventilation plotted against the reaction finally becomes a straight line parallel to the abscissa. That is, the centre is no longer stimulated by increase in the reaction of the blood and the stimulus maintaining respiration in this condition is some other than *H*-ion or HCO_3 or CO_2. The nature of this stimulus we are endeavouring to discover. The apparent shift of the apnœic threshold in 2 above is, we think, due to the appearance of this stimulus before the sensitivity of the centre to *H*-ion is reduced to zero.

Ether gives similar changes to the above but the accurate measurement of the reaction is impossible because of the unknown errors introduced into the gas analyses by ether vapour.

A respiratory waistcoat. By B. A. McSwiney.

This waistcoat was devised for measuring the expansion of the different regions of the chest wall. The waistcoat is made in two pieces, front and back, and is fastened together by tapes or straps at the shoulders and sides. A number of small balloons have been placed in the waistcoat so that they lie over the different areas of the chest wall.

To measure the expansion of any area of the chest wall, the balloons on the right and left side are connected by two pieces of rubber tubing, to a bellows and an oil manometer. The balloons are blown up, the oil in the tube of the manometer being always raised to the same height. The variations in the height of the oil in the manometer registers the expansion of that area. To compare the expansion of one side of the chest wall with the other, one of the rubber tubes is clipped. Records of the movements are obtained by connecting the manometer with a tambour and lever, writing on smoked paper, or by connecting the manometer with an optical tambour.

Measurement of the capillary (arteriole) pressure in man. By Leonard Hill and James McQueen.

The method of v. Kries, variously modified as it has been, gives the pressure required to blanch an area of the skin. The resistance of the skin to deformation introduces an unknown error. Moreover, as the pressure which blanches the skin stops the circulation it measures, not the capillary pressure, but that of the arterioles which supply the compressed part. To measure the resistance of the skin to deformation and so arrive at the true arteriole pressure we proceed as follows. A fine jet of water at body temperature is allowed to play on the skin, *e.g.* behind the thumb nail, and the pressure found which just pales the skin as the jet is slowly moved to and fro. The hand of the subject is held at the level of the heart while the jet plays upon it. The pressure is measured by turning the jet so that it plays vertically up alongside a scale.

The hand is next raised to empty the veins, and the cuff of a sphygmomanometer, previously placed on the upper arm, is then connected with a cylinder of compressed air so that the brachial artery is rapidly occluded. There is no pressure now in the capillaries. This is shown by the fact that any large area on the hand from which the blood is expressed does not refill. The jet of water is again found which just expresses the blood from behind the finger nail, and the height of this jet measured.

This reading is deducted from the first reading and the difference gives the true arteriole pressure. In order to keep enough blood in the capillaries it is advisable to put a band round the wrist, and remove this just before making the second reading.

Example. The height of the jet in the first case was 34 cm. H_2O, in the second case 22 cm. H_2O—difference 12 cm. H_2O.

The true arteriole pressure comes out at about 10 mm. Hg.

Basler[1] made a small cut in the skin and connected the bleeding part with a manometric arrangement. He thus found a pressure of 7–9 mm. Hg, a good agreement.

Conduction of sensory impulses from the bladder by the inferior hypogastrics and the central afferent connections of these nerves. By George Riddoch, M.D., M.R.C.P.

The accidents of the recent war have afforded further evidence to show that the inferior hypogastric splanchnic nerves conduct sensory impulses from the bladder to the spinal cord. In this preliminary note the evidence is briefly summarised.

Bilateral complete lesions of the cauda equina involving the last four sacral posterior roots abolish conduction of afferent impulses by way of the pelvic nerves from the bladder and the pudic nerves from the penis. The following facts however show that in such cases vesical sensibility is not gravely disturbed. (1) The penis is entirely anæsthetic and the passage of a catheter along the urethra fails to evoke a response. But as soon as the bladder is penetrated and the point of the instrument touches its wall in the region of the trigone the patient recognises that something has happened and refers the sensation to a point deep in the pelvis. (2) He is at once aware that his bladder is full and has a desire to micturate. If involuntary micturition has become established, the contents of the bladder will now be evacuated and the mild discomfort will disappear. If, however, retention is still present, as it is for at least the first few months after the injury, and the urine is not withdrawn artificially the discomfort increases in intensity and is now largely referred to the lower abdominal wall over the pubis. There can be no question at this stage of stimulation of the somatic sensory nerves by stretching the abdominal wall for the bladder may only reach a little above the brim of the pelvis. Sometimes if the call to urinate is still neglected the discomfort after a time diminishes and may almost disappear. This is due to a new postural length of the visceral muscle having been adopted and the organ accepts an additional quantity of urine without undue tension on its walls. (3) More usually however the sensation of discomfort gradually becomes more acute as distension of the bladder increases. The patient is doubled up with pain which he refers to the pubic and inguinal regions. The pain is of the particularly disagreeable kind that is associated with abnormal tension of the walls of hollow viscera. It may be accompanied by rigidity of the abdominal muscles and tenderness of the skin and deep tissues in the neighbourhood of the pubis and groins. (4) When severe cystitis is a complication attacks of pain of the same nature are apt to occur although there may be no overdistension of the bladder. In such cases the pain is probably evoked in an intense form by periodic spasms of the vesical muscle brought on by the inflammatory process for it is relieved by drawing off the contained urine.

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[1] Cited by Tigerstedt, *Ergebnisse der Physiologie*, p. 13. 1920.

These sensations of fullness, discomfort and pain must be excited by impulses which travel in the inferior hypogastric nerves. They still occur when all the posterior spinal roots below the third (possibly the second) lumbar are destroyed. On the other hand vesical sensibility is entirely abolished by a severe lesion of the spinal cord at the level of the eleventh thoracic segment. Hence, the main central connections of the afferent fibres of the inferior hypogastric nerves lie between the eleventh thoracic and the third lumbar segments of the spinal cord.

Class exercises on blood reaction. By C. Lovatt Evans.

The following instructive experiments can be easily carried out by Senior students, or shown as demonstrations. The reactions of the blood and bicarbonate solutions are determined by the colorimetric method described by Dale and Evans(1).

1. *Reaction of aqueous carbon dioxide* (without buffers).

Distilled water free from carbon dioxide has a p.H in the neighbourhood of 7·0. Air-saturated water (which contains about 0·3 c.c. CO_2 per litre) has a p.H about 5·5; water saturated with alveolar air, and containing about 45 c.c. CO_2 per litre, a p.H of about 4 (acid to methyl red).

2. *Reaction of bicarbonate*-CO_2 *solutions.*

(i) (Doubling the $NaHCO_3$ concentration results in halving of the C_H, *i.e.* in a rise of 0·3 in *p*.H.) Prepare solutions of ·01, ·02, and ·04 *M*. $NaHCO_3$ and saturate 5 c.c. of each solution with alveolar air in a separating funnel at room temperature. Dialysis is unnecessary, the mixture being transferred directly to a comparator vessel and covered with paraffin.

(ii) (Proportionate increase or diminution in the concentration of both CO_2 and $NaHCO_3$ leaves the *p*.H unchanged), *e.g.*

$$\left.\begin{array}{l}\cdot01\ M.NaHCO_3 + \ \ 3\ \%\ CO_2\\ \cdot02\ M.NaHCO_3 + \ \ 6\ \%\ CO_2\\ \cdot04\ M.NaHCO_3 + 12\ \%\ CO_2\end{array}\right\}\text{ have the same } p.\text{H.}$$

For this demonstration, the required mixtures of CO_2 and air can be made up in a flask as recommended by Bayliss(2). As small a volume of the solution and as large a volume of gas as possible should be used, in order to ensure that the final concentrations of CO_2 do not materially differ from those originally made up. Analysis of the gas after attainment of equilibrium is, if practicable, to be preferred.

3. *Reaction of blood at alveolar* CO_2 *tensions.*

Four or five c.c. of fresh oxalated blood (human preferred) is placed in a separating funnel filled with alveolar air and kept at 37° by immersion in a water bath, while the funnel is rotated horizontally for 5 minutes. Transfer direct to a dialyser and carry out dialysis and titration as described by Dale and Evans. If a series of observations at different CO_2 tensions be carried out, *e.g.* by different members of the class on the same blood, a CO_2 reaction curve can be constructed. Phenol red is to be preferred to neutral red for blood dialysates having a *p*.H above 7·8.

4. *Relation of blood reaction to alkali reserve of plasma.*

(The reaction of blood is chiefly determined by the bicarbonate content and CO_2 tension of its plasma.) Divide 15 c.c. of oxalated blood into three equal parts. To the first add 1 c.c. of ·85 p.c. salt solution, to the second 1 c.c. of ·2 *M*. $NaHCO_3$ and to the third 1 c.c. of ·05 *N*. HCl. Each sample is then saturated with alveolar air (at room temperature) and the *p*.H determined after dialysis as before. The second and third blood samples will illustrate the effect of increased and diminished alkali reserve respectively.

5. *Effect of temperature.*

(Blood at a given CO_2 tension is more alkaline at a higher than at a lower temperature.) Determine the *p*.H of blood saturated with alveolar air at room temperature and at 37°.

(1) Dale and Evans. *Journ. Physiol.* 54. 167. 1920.
(2) Bayliss. *Journ. Physiol.* 53. 166. 1919.

Cooling and warming of the body by local application of cold and heat. By Leonard Hill.

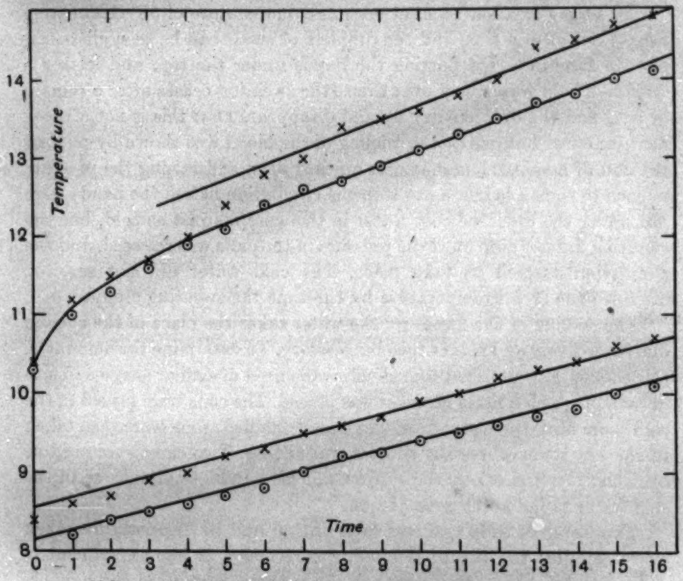
On sitting in a hot room at constant temperature of 38° C. sweating breaks out after a time and the droplets of sweat can be seen glistening on the forehead. On putting the hands under the tap, and letting a stream of cold water play over them, the sweating ceases after a minute or two, and the droplets dry up and disappear. That this is not a reflex nervous effect but one due to cooling of the blood was shown by putting the cuff of a sphygmomanometer on each arm, and raising the pressure in each to such a height as to stop the circulation before the hands were put under the tap. The cold water in this case *felt* just as cold, but the sweating did not stop until the pressure in the cuffs was released, and the circulation allowed to take place. The cold water did not sensibly diminish the rectal temperature by the time the sweating stopped.

The cooling of the hands by the water takes the place of the cooling due to sweating and makes this unnecessary. To determine the amount of this cooling four polished tinned cans were used of similar shape and size in each of which 5 litres of water was placed. The cans were placed in the cold room until the water in all was equally chilled; they were then taken to the hot room where the subject was sitting. Two cans were used as controls; into the others the subject put his hands, sinking these in the cold water as far as the wrist joints.

The water of each can was kept stirred and its temperature taken every minute: the rise of temperature recorded in one experiment is shown in the graph. While that in the controls is uniform throughout, that in the cans containing the hands, at first more rapid becomes uniform after the fifth minute. The uniform rate of heat loss from the hands is shown in the first four observations given in the table.

Subject	Temperature of room	Initial temperature of water	Total heat loss of hands in kilo-calories per min.	Total heat gain of hands in kilo-calories per min.
M.B.	39° C.	4 °C.	1·2	—
C.P.	39	10·4	0·6	—
M.B.	17	8·3	0·46	—
C.P.	17	9·8	0·64	—
M.B.	17	42·5	—	0·4
C.P.	17	39·9	—	0·16

Two of these were carried out in the hot room and two at ordinary room temperature. The last two observations in the table show the gain of heat occasioned by placing the hands in hot water. The experiments show how effectively the heating or cooling of a small portion of the body can heat or cool the whole. In experiment 1 the rate of loss of heat was no less than 72 kilo-calories per hour—the total heat production of a resting man.

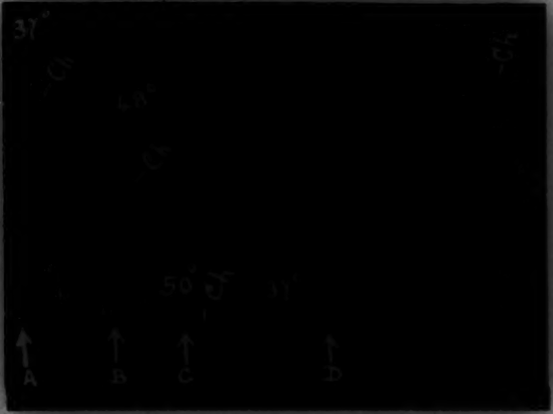


10 litres rose 0·6° C. in 10 minutes due to the immersion of the hands.
Heat loss = 0·6 kilo-calorie per minute.

I am indebted to Miss Hargood-Ash for assistance in carrying out this research and to Miss Brad and Mr Pergande who acted as subjects.

Heat paralysis in plain muscle. BY C. LOVATT EVANS.

When the oxygenated Ringer's solution, in which an isolated guinea-pig's uterus is suspended, is gradually heated, the small spontaneous contractions of the plain muscle disappear suddenly at about 40–50° C. The tissue now fails to respond to even large doses of histamine, and might be supposed to be dead. But if re-cooled to body-temperature it will be found to have regained its excitability, though the contractions are slower and often smaller than before (Fig. 1). The condition at 49° thus closely resembles that of heat paralysis studied in the central nervous system, and in primitive animals(1).



Contractions of guinea-pig uterus in oxygenated saline. Frontal writing. Time = minutes. At *A*, 0·05 mg. histamine: temp. = 37°. At *B*, 0·05 mg. histamine: temp. 48°. At *C*, 0·1 mg. histamine: temp. = 50°. At *D*, 0·05 mg. histamine: temp. = 37°. (A uterus made inert by CO_2 and lactic acid showed a similar paralysis and recovery.)

If heated for several minutes at 50° the plain muscle is killed: its death occurs while it is still fully relaxed, and is hence not comparable with the heat-rigor in skeletal muscle. On further heating a heat-coagulation, having no relation to the death-changes, sets in at about 63°. The lactic acid content of the heat-rigor muscle is greater than that of the resting muscle, but both are small.

Determinations (by the microrespirometer) of the oxygen consumption of uteri at different temperatures show that this is maximal at about

40–45°, *i.e.* a little below the temperature at which the heat paralysis is present: a further rise of temperature irreversibly reduces the oxygen intake.

Oxygen Usage of Guinea-pig Uterus.

Temp. °C.	c.c. O_2/gm./hr.	Temp. °C.	c.c. O_2/gm./hr.
14·0	·073	40	·617
25	·236	45	·617
30	·35	48	·585
35	·483	48	·538
37	·545	37	·330

If oxygen lack plays any part in causing the heat paralysis, it must be a purely relative lack, *i.e.* the oxygen requirement of the tissue (whatever that term may mean) is greater than the supply of oxygen.

In explanation of these results it seems reasonable to suppose that metabolites are produced in the resting tissue at a rate which is accelerated by rise of temperature. These are removed partly by diffusion and partly by oxidation. When accumulation occurs, the tissue is inexcitable, and further accumulation kills the tissue.

I have previously shown (2) that plain muscle contracts perfectly well at ordinary temperatures in absence of oxygen, but that this anaerobic contraction cannot be sustained. Significantly enough, lactic acid and carbon dioxide can cause a similar relaxation of the guinea-pig uterus, which is then less responsive or quite inert to histamine.

If the above hypothesis is the correct one, heat paralysis should occur at a lower temperature in the absence of oxygen; this was found to be the case, paralysis coming on at about 47–48° when nitrogen was bubbled into the bath instead of oxygen: subsequent recovery was, however, incomplete.

These experiments appear to be in complete agreement with those of Winterstein (3), who also advances similar explanations.

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(1) Winterstein. *Ztschr. f. allg. Physiol.* 1. 129. 1902.
(2) Evans. *Journ Physiol.* 53. 17. 1919.
(3) Winterstein. *Ztschr. f. allg. Physiol.* 5. 323. 1905.

A differential balance for use with Martin's bicycle ergometer.

By E. H. J. Schuster, D.Sc. (*Medical Research Council*).

This instrument is designed for use with Martin's bicycle ergometer on which work is done by overcoming the frictional resistance of a brake band applied to a flywheel mounted in place of the rear wheel of the bicycle. In the usual pattern of machine a cord is led from each end of the brake band by a suitable train of pulleys to a spring balance mounted in a convenient position, and the frictional resistance is indicated by the difference of the readings of the two balances. The differential balance takes the place of both spring balances and it contains in itself the contrivance by which the desired resistance may be attained by adjusting the tension on the cords. The principal advantage claimed for it is that it gives a direct measure of the difference in the pull of the two cords, and that in this way the frictional resistance may be more easily ascertained and adjusted.

The instrument consists of a plate (*A*) sliding on a guide *B* which is attached by means of the lugs (*B'*) to a vertical post in front of the handle-bar of the bicycle. The position of *A* on *B* may be adjusted by means of the screw (*C*) actuated by the knurled head (*C'*). If *C'* is turned in a clock-wise direction, *A* is raised and the tension of the cords consequently increased.

The dial (*D*) is rigidly attached to the plate *A* by means of four stout steel studs (not shown in the figures). From the centre of *D* a fifth steel stud, of small diameter, runs backwards at right-angles to *A*, and this forms the pivot of the balancing member.

The balancing member consists of a sleeve (*J*) carrying in front a grooved pulley (*F*) (that part of *F* which lies behind the dial is indicated by broken lines in Fig. 1), and behind at right angles to its axis a spindle (*G*). The lower part of *G* is threaded, and on to it a cylindrical bob (*H*) is screwed, which may be locked to it at any desired position by the back nut (*K*). The balancing member is free to rotate as one piece about its pivot.

A continuous cord *N* passes round the groove in the pulley and is kept from slipping by a little clamping screw borne by the angle-piece *M*. The two ends of the cord are attached to the two ends of the brake band, being led to them in the usual manner by a train of pulleys.

The action of the flywheel on the brake band, when the ergometer is in use increases the pull of the cord on one side of the pulley *F* and decreases it on the other side. The pulley is thus caused to rotate and the bob (*H*) to swing out until it reaches a position in which it balances the difference of tension of the two ends of the cord. This position can then be read on the scale engraved at the circumference of the dial by means of the pointer (*L*), which is attached to *F* and is bent in such a way as to pass through the slot (*E*) in the dial.

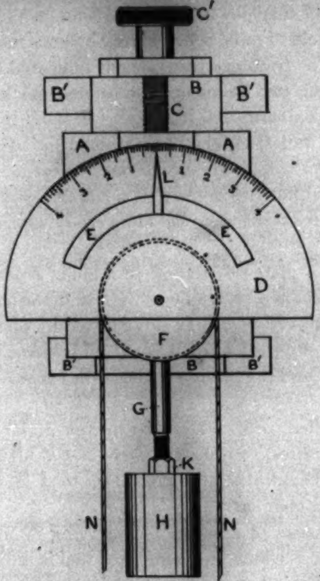

Fig. 1. Front elevation × ½.

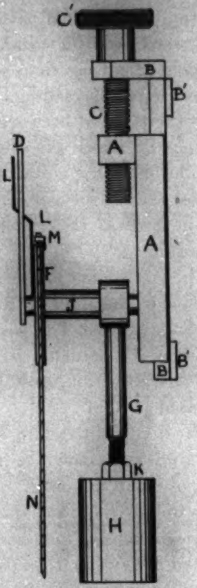

Fig. 2. Side elevation × ½.

The successive graduations on the scale from 0–4 in either direction represent angles whose sines increase by equal increments, *i.e.* the sine of the angle 0–1 is ½ of the sine of the angle 0–2 and ⅓ of the sine of the angle 0–3, etc. What unit of weight is represented by each division depends on the weight of the bob *H* and its distance from the centre of rotation relative to the effective diameter of the pulley *F*. In the instrument exhibited the reading is in kilograms. The bob *H* weighs approximately 1 kilo. It need not be made to an exact weight as the correct reading of the instrument may be secured by screwing the bob up or down on the spindle *G*.

Anaphylaxis and immunity "in vitro." By H. H. DALE and C. H. KELLAWAY.

According to the view which has been advocated by the late R. Weil and by one of us, the difference between anaphylaxis and true immunity depends on the distribution of an antibody, probably a precipitin, between the cells and the body fluids. In anaphylaxis the antibody is supposed to be predominantly located in the cells, where its union with the antigen, when this is injected, causes the anaphylactic "shock"; in immunity it is supposed that a predominance of antibody in the circulation fixes the antigen before it can reach the cells. According to the alternative view, the anaphylactic shock is due to the liberation of a poison in the blood, brought about by the interaction there of antibody and antigen. Weil showed that introduction of excess of antibody, into the circulation of an anaphylactic animal, prevented the appearance of shock when the antigen was injected immediately afterwards. This phenomenon, which seems to be decisive in favour of the dependence of anaphylaxis on cellular predominance of antibody, can be demonstrated *in vitro*.

A guinea-pig is rendered highly sensitive to crystalline egg-albumin by injecting a small dose (*e.g.* 0·5 c.c.) of serum from a rabbit immunised against that protein, and leaving a few days for the disappearance of the antibody from the circulation and its fixation by the body cells. The guinea-pig is then killed and the uterus perfused free from blood with warm Ringer's solution. One horn of the uterus, suspended as usual in oxygenated Ringer's solution, responds to egg-albumin in a very low concentration (1 in 1 to 10 millions). Thereafter it is completely insensitive to a renewal of such concentrations of the antigen. The other horn is then suspended in the Ringer's solution, to which is added about 1 part in 40 of the same rabbit's antiserum, as was used to produce the "passive" anaphylaxis. In this solution the horn of the uterus responds not at all, or very weakly, to the doses of egg-albumin which caused prompt and large contraction of the first horn. It neither reacts, nor is it desensitized; for when the serum dilution is replaced by pure Ringer's solution, and the second horn again tested with egg-albumin, it responds at least as well as the first. Antibody in the plain muscle cells renders them acutely sensitive to the corresponding antigen; excess of antibody in the fluid bathing them protects them from the antigen, though they are still sensitive. The conditions correspond to anaphylaxis and immunity respectively.

The pressure in the small arteries, veins and capillaries of the bat's wing. By Leonard Hill.

An area of the second segment of the bat's wing was placed on the apparatus of Roy and Graham Brown, so that a main arterial branch and the arterioles coming off from it at right angles, together with the vein, venules and capillaries, could be compressed and microscopically observed.

The main arterial branch measured about ·08 mm. in diameter and the arterioles about ·015 mm. The bats were at first hybernating and cold, but owing to handling became roused up and warm-blooded; they were put under urethane anæsthesia. The pressure required to stop the flow in the main arterial branch rose from about 20 mm. Hg when the bat was cold to 50 mm. Hg when the bat was warm. When the systolic pressure in the main arterial branch was about 50 mm. Hg that in the arterioles was about 15 to 20 mm. Hg. A momentary application of pressure of less than 1 cm. H_2O momentarily checked the venous flow.

A momentary compression of 1 to 2 cm. H_2O momentarily checked the flow in the capillaries. A compression of 2–3 mm. Hg sufficed to slow the flow in the capillaries where the velocity of flow was least. There is then a great drop of pressure on passing from the main arterial branch to the arteriole, and while the capillary pressure is very low, that in the vein is only just positive.

A slow, rhythmic pulsation, as is well known, helps to maintain the flow in the veins of the bat's wing. This is little evident in the hot-blooded state when the velocity of flow in the arteries and veins is very fast.

Owing to the rapid beat of the heart no pulsation is seen on compression; the flow remains continuous until it stops.